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Fluorosis in Cattle and Sheep

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THE UNIVERSITY OF TENNESSEE
AGRICULTURAL EXPERIMENT STATION

KNOXVILLE

Foreword

From Tennessee farmers came complaints of injury to their crops and livestock. It seemed probable that such injury resulted from fluorine-bearing effluents from nearby manufacturing operations in two locales. Because of the seriousness of the problem, The University of Tennessee Agricultural Experiment Station initiated a project to identify the causes for, and extent of, injury to crops and livestock and to explore remedial possibilities.

The findings reported in this bulletin represent a part of the Animal Husbandry phase of a joint project that was initiated in 1947 by the Departments of Chemistry and Animal Husbandry–Veterinary Science on "Effects of Certain Atmospheric Effluents Upon Plant and Animal Life at Two Locales in Tennessee." This study, which involves several groups of cattle, is being continued and additional reports will be published. A companion bulletin reporting the Chemistry phases is in preparation.

The research work now reported, in part, was made possible through State and Federal funds, supplemented substantially by grants-in-aid from the Aluminum Company of America, Monsanto Chemical Company, Victor Chemical Works, and Tennessee Valley Authority. The collaboration and financial support from those corporations is of inestimable value to a successful furtherance of this research.

John A. Ewing
Assistant Director

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General Summary

Cattle and sheep have been used to study the physiological effects of various levels of fluorine fed to cattle (from control up to 1200 parts per million F added as sodium fluoride) and to sheep (from control up to 200 ppm F as NaF) upon: (1) feed consumption and utilization; (2) weights and gains; (3) reproduction and calf records; (4) urinary fluorine; (5) bone storage or changes; (6) blood; (7) teeth; and (8) general condition.

Groups of cattle were fed hay, and others grazed pastures and were fed hay contaminated with varying amounts of fluorine from an aluminum smelting plant. Numerous studies were made, employing rats and rabbits as pilot animals for cattle and sheep studies, to evaluate the relative toxicity of various fluorine sources and to try to find materials that could be added to rations to counteract (alleviate), totally or in part, the effects of increased amounts of ingested fluorine.

Nomenclature and classification for incisor teeth and techniques that can be used to evaluate the occurrence and extent of dental fluorosis are presented. Consideration was given to physiological changes associated with various levels of F intake in an effort to formulate a basis for the establishment of a maximal level of fluorine that can be tolerated by cattle and sheep.

Studies with yearling heifers, receiving 10 to 1200 ppm F added as NaF to control ration, or ingesting hay or hay-and-pasture forage contaminated with F from an aluminum smelting plant and with sheep fed rations containing up to 200 ppm F as NaF provided data which indicated the following:

Feed Consumption

1. Cows receiving rations with fluorine added as NaF at 10, 20, and 30 ppm (total of 17, 27 and 37 ppm F in ration) or cows grazing pastures that averaged up to 44.9 ppm F and fed hay that averaged 51 ppm F for a four and one-half year period showed no significant difference in feed consumption.

Ewes fed control rations with fluorine as NaF added up to 100 ppm showed no appreciable decrease in feed consumption by the end of three years on test. The wether lambs receiving rations with 200 ppm fluorine added as NaF for five months showed an indication of a slight decrease in feed consumption, while wethers on lower levels did not.

Results with laboratory animals and cattle indicate that age of animal at time of initial ingestion and source of fluorine may affect the results obtained.

2. Cows in Experiment I, ingesting rations containing 40 and 50 ppm F (total of 47 and 57 ppm F), showed a significant decrease in feed consumption for the period from two and one-half to three and one-half years on test, but not before this period. This reduction in feed consumption continued for the period reported. However, the same levels of fluorine in rations fed cows in Experiment II had not shown a decrease up to two years on test.

3. Cows ingesting rations containing 70 and 100 ppm of added F showed a significant decrease in feed consumption within 18 months from initiation of the experiment.

4. Heifers ingesting rations containing 200 and 300 ppm of added F revealed a significant decrease in feed consumption within 12 months from start of the test.

5. Heifers ingesting rations containing 600, 900 and 1200 ppm F added as NaF exhibited impaired appetite within three days, and the fluorine had to be administered by capsule to insure consumption. Heifers on these levels practically starved to death.

6. The addition of 0.5 percent defluorinated rock phosphate in rations containing an added 100 ppm fluorine as NaF improved feed consumption and calf production.

Weights and Gains

Cows in these tests receiving rations with increased fluorine content, from 200 up to 1200 ppm, compared to control animals, showed within 420 days from start of the test a decrease in body weight directly related to level of fluorine intake.

Considering the reproduction and calf records there was no appreciable difference in weights and gains between any of the groups either in barn feeding or pasture tests receiving less than 70 ppm fluorine added as NaF. This includes three barn groups receiving 50 ppm of added fluorine (total 57 ppm) and the highest pasture lot which ingested contaminated pastures that averaged 44.9 ppm fluorine and hay that averaged 51 ppm for a four and one-half year period (weighted over-all average of 47 ppm F). If differences in calving and calf production are evaluated, then the cows, receiving 70 ppm and 100 ppm F added as NaF, compared to control cows, probably showed a significant decrease in weights and gains.

Cows ingesting F on forages contaminated by fluorine from an aluminum smelting plant, compared on the basis of either ppm in feed or mg./kg. of body weight ingested, indicated that the F from NaF is as toxic, and probably more toxic to cattle, as F in contaminated forages from an area near an aluminum smelting plant.

Ewes ingesting rations containing up to 100 ppm fluorine added as NaF over a three-year period showed no differences in final weight compared to ewes ingesting control rations.

In a 140-day feeding trial, lambs fed rations containing fluorine added as NaF up to 200 ppm indicated that the addition of 200 ppm resulted in a decrease of body weight; those on lower levels did not.

Reproduction and Calf Records

Under the conditions of these tests fluorine did not affect reproduction directly in cattle receiving up to 1200 ppm fluorine or ewes receiving up to 100 ppm fluorine in rations. But, when the level of fluorine was increased to a level where it materially interfered with feed consumption and general condition, as shown by heifer groups continuously ingesting 70 and 100 ppm fluorine as NaF for over 18 months, there was a lowering of reproduction and calf records.

Digestion and Balance Studies

Under the conditions of Experiments I and II with cattle and Experiment X with wether lambs, fluorine levels fed up to 100 ppm added fluorine as NaF did not significantly affect the apparent digestibility of the rations.

With cattle and sheep the fluorine stored in the body was directly related to the level of intake as shown by the fluorine balance studies.

Although there were differences in calcium, phosphorus, and nitrogen balance studies with cows and with wether lambs, no significant differences occurred that could be associated with the levels of fluorine fed, except for an indication that at the higher fluorine levels ingested, phosphorus may be tied up.

The feeding of 0.5 percent aluminum sulfate in rations containing up to 50 ppm F for cattle and aluminum sulfate or aluminum chloride in rations containing up to 100 ppm F for wether lambs showed that the soluble aluminum compound tied up to 20 to 40 percent of the added fluorine in the feces and prevented its absorption as evidenced by increased F content of feces, and decreased urine F content, in comparison to animals receiving the same level of increased fluorine without the alleviator in their rations.

Fluorine Content of Bones

Fluorine content of leg, jaw, or rib sections taken by biopsy of cattle or bones of sheep, rats or rabbits was related to level of fluorine ingestion and length of time the given levels were ingested.

The addition of aluminum sulfate to rations containing increased levels of fluorine for cows, ewes, lambs and rats, compared to animals receiving the same ration without the added aluminum sulfate, decreased the fluorine content of the bones and effects on developing teeth. Results showed that the extent of alleviation is dependent upon the solubility of aluminum material and the level of fluorine fed.

Blood Studies

Studies with cattle and sheep indicate that the fluorine content of feed, up to 200 ppm fluorine, did not affect the phosphorus, calcium or hemoglobin content of the blood.

Urinary Fluorine

The fluorine content of the urine varied generally with the level of intake. Results show that urine analyses should be used only in conjunction with other criteria in diagnosing or determining the extent and severity of fluorosis. Urine samples should be secured from several animals in a group and, where possible, at periodic intervals to give the most accurate results.

The occurrence and degree of fluorosis in cattle depends upon many factors, including: (1) The level of fluorine ingested. (2) The age of the animal and the stage of tooth development. (3) The length of time exposed to the increased fluorine ingestion. (4) The initial fluorine stored in animal body. (5) The solubility and availability of the fluorine material ingested.

Introduction

The fluorine problem in livestock production is one of increasing importance. Fluorosis or fluorine toxicosis has been commonly defined as "chronic poisoning with fluorine." To some, the word fluorosis may mean the condition that develops when ingestion of fluorine is sufficient to cause physical changes, while to others, the word may mean the condition resultant from any ingestion of fluorine above normal levels. Since so-called "normal" levels may vary considerably and since results from fluorine ingestion just above these levels are practically indistinguishable from normal levels, the term fluorosis in cattle and sheep probably should be confined to mean, at least, "chronic fluorosis," wherein the ingestion of fluorine is sufficient to produce some definite gross pathology associated with the condition.

Fluorosis is not an infectious disease; it is an abnormal physiological condition. Factors governing specific effects that may develop from continued fluorine ingestion include: (1) the age of the animal when excessive amounts of fluorine were ingested, and the stage of tooth development; (2) the level of fluorine ingested; (3) the length of time of increased fluorine ingestion; and (4) initial amount of fluorine stored in body.

Fluorosis in livestock may develop through several channels, one of which is the use of mineral supplements containing fluorine. Such minerals include rock phosphate, which contains 3 to 4 percent fluorine; and phosphatic limestones, which contain fluorine in proportion to the amount of phosphorus present. The fluorine content of some defluorinated rock phosphates commonly used in mineral supplements sometimes may constitute a considerable portion of the total fluorine ingested.

Chronic fluorosis in farm animals, both in this country and abroad, has been reported in areas adjacent to industrial plants emitting fluorine-containing gases and dusts. Livestock ingest the fluorine by grazing forages or consuming hays on which these gases and dusts have settled. Emissions of fluorine occur in the production of acid phosphate and defluorinated phosphate, in the smelting production of aluminum, in the manufacture of bricks from fluorine-bearing clays, in calcining of ironstone, and in certain enameling processes. Fumes from coal-burning furnaces also may contribute to the industrial hazard, since coal and shales associated with coal contain from 40 to several hundred parts per million fluorine, according to Churchill *et al.* (1948).

Fluorine is very active chemically but does not occur in a free state. Fumes from factories that process fluorine-containing materials may be largely hydrofluoric acid; and fluorine-bearing dusts may consist of fluorides, such as sodium fluoride or cryolite, which have been volatilized and then condensed in the surrounding air. Vegetation may absorb the gas and retain some of the dust on plant surfaces. The question of whether the material is deposited only on the plant surface or is absorbed and subsequently incorporated into the plant has not been satisfactorily answered. The extent of forage contamination therefore, depends upon several factors, such as the amount of fluorine-containing materials emitted, weather conditions, prevailing winds, topography of surrounding terrain, and type of vegetation.

Water contributes significantly to the fluorine hazard when its fluorine content attains levels of several parts per million. Fluorotic conditions ascribed to high-fluorine water have been reported from Australia and India; and from Texas, Colorado, Arizona and other states.

Fluorotic conditions have been reported that were caused by dusts and gases from volcanic eruptions. During eruption, the fluorine-bearing gases and ashes flow out of the volcanic opening in tremendous quantities.

MacIntire *et al.* (1942, 1947, and 1951) have shown that accumulations of calcium fluoride in the soil exert no harmful effects upon plant growth; and, where soils are adequately supplied with calcium, there is relatively little uptake of fluorine by the common grasses and legumes grown in Tennessee. However, depending upon the density and height of vegetation and soil conditions, rain waters may splash considerable phosphatic soil upon the vegetation which may be grazed by animals. Likewise, depending on conditions, dust of high phosphate soils or fertilizers containing high phosphate materials, may be on vegetation ingested by animals. Thus, cattle grazing where top soils contain thousands of ppm of fluorine may develop chronic fluorosis. It is the total amount of available fluorine ingested by animals that must be considered in the diagnosis and extent of fluorosis.

Information concerning amounts of fluorine that would be damaging when consumed over an extended period would be of immeasurable value to the livestock producer and feeder. Also, some materials that could be fed or administered to livestock to alleviate a part or all of the effects of fluorosis would be of great value to agriculture and industry.

Relatively few extended feeding experiments have been conducted with cattle and sheep consuming known levels of fluorine. Information concerning the amount of ingested fluorine necessary to be damaging over an extended period of time, therefore, would be of great economic and practical importance to the feeder and producer of livestock. The fluorine levels used in most of the studies reported herein were selected in an attempt to determine the chronic toxic levels that might be encountered in areas where livestock have developed chronic fluorosis.

Likewise, evaluation of materials which might prevent or alleviate effects attributed to fluorosis would be of value to livestock producers in areas where livestock have developed chronic fluorosis.

The present studies at this Station are concerned with the effects of feeding various levels of fluorine from several sources to cattle and sheep in an effort to establish the chronic toxic levels that can be tolerated by cattle without material damage, and the effects and symptoms on cattle fed chronic to acute levels of fluorine.

Review of Literature

Interest in fluorosis has been stimulated by the recognition that certain functional disabilities suffered by livestock and by man are due to ingestion of excessive amounts of fluorine. Occurrence of chronic fluorine intoxication have been described in flocks and herds grazing in many parts of the world. Symptoms of fluorosis have been reported among animals grazing in the vicinity of certain processing operations, superphosphate plants, aluminum plants, brick kilns, steel production centers and elsewhere.

In Iceland, as early as 1100 A.D., attention was called to a disease of domestic animals, which appeared particularly in grazing sheep after volcanic eruptions (Roholm, 1937). The symptoms described were similar to those in cases later diagnosed as fluorosis. As early as 1670 it was known that glass was attacked by the fumes produced when fluorspar was treated with sulfuric acid, and in 1802 Morichini used the glass test to determine the presence of these same "fumes" in substances of animal origin. As a result of these studies, fluorine was detected in fossil teeth. Later, (1805), Gay-Lussac and Berthollet found fluorine in enamel of normal teeth and thus raised the question concerning the extent and significance of the occurrence of fluorine in teeth and other body substances. The presence of fluorine has been reported in the ash of blood and of milk, in the brain, yolk and shell of the egg, and in various organs and tissues of the animal body. A summary of literature on the fluorine content of foods and beverages is given by McClure (1939).

Fluorosis has been reported as occurring in grazing animals; from feeding mineral supplements containing excessive amounts of fluorine; from drinking fluorine-contaminated water (Rand and Schmidt, 1952) and in animals grazing on phosphatic limestone soils, especially where the phosphatic rock appears near surface levels (Phillips, 1952).

Chronic fluorosis in cattle has been produced experimentally and reported by several investigators: Reed and Huffman (1930), Phillips *et al.* (1934), Elmslie (1936), Majumdar (1943) and Hobbs *et al.* (1953).

A voluminous literature has resulted from the numerous investigations which, for the most part, have been devoted to the study and description of symptoms, relative toxicities of different fluorine compounds and quantities required to bring about gross changes in the different animal species. Information describing the details of toxic effects of fluorine on the different tissues and organs of the animal body is reviewed by Dean (1936), McClure (1933), Peirce (1939), DeEds and Thomas (1933), Gettler and Ellerbrook (1939), and Greenwood (1940). Mitchell and Edman (1952) presented an extensive summary of literature concerned with the fluorine hazard in livestock feeding, with special emphasis on the relative toxicities of the various fluorine compounds. One of the most complete reviews of the basic literature on fluorine intoxication is the monograph by Roholm (1937).

In current literature few reports distinguish between symptoms of acute and chronic fluorosis. Some have described symptomatic fluorosis that is unquestionably complicated by other factors. This has led to confusion and many times to the misinterpretation of the specific syndrome characteristics of fluorosis itself. A critical study and review by Schmidt and Rand (1952) on symptoms of chronic fluorosis, clinical findings, morbid anatomical and biochemical findings under field investigations and in controlled feeding experiments, has aided in clarifying this subject. Additional detailed information on the fluorine problem may be obtained from the following reviews: Roholm (1937), Schmidt and Rand (1952), McClure (1933), Peirce (1939), DeEds and Thomas (1933), Greenwood (1940), Mitchell (1951), Mitchell and Edman (1952), and Smith (1951).

Because of the importance of dental changes in the determination of the effects of ingested fluorine, it appears worthwhile to review briefly some of the literature dealing with the specific effects of fluorine on the teeth.

According to Dean (1936) the first reference to mottled enamel was made by Eager in 1901. McKay and Black (1916) reported mottled enamel as a water-borne disease. In addition to 27 references to literature dealing with dental fluorosis in man, Dean cites three references to reports on an analogous condition in domestic animals. One reference is made to an article by Dean (1935) on mottled enamel in teeth of cattle. Some of the earliest information regarding the relationship of fluorine to dental changes in cattle and sheep came from French workers in North Africa, particularly Velu (1931), and Balozet (1934). The occurrence of mottled enamel in the permanent teeth of cattle from drinking artesian well water high in fluorine in South Carolina was reported by Dean (1935).

Velu reported that the temporary teeth showed little or no effect, while the permanent incisors were definitely marked. The surface was lusterless and speckled with brown. The enamel was absent in places, exposing the dentine. The teeth were low and stump-like, and the sharp edge had disappeared. Spacing between the teeth may have been increased and the molars showed excessive, irregular wear.

The role of fluorine as a cause of mottling of enamel in human teeth was reported by Smith and Lantz (1931). Schour and Smith (1934) reviewed the histological effects of fluorine-containing diets on the dental tissues of the rat. They also reported results of their own experiments where fluorine was fed to or injected into rats. Their findings, as a whole, agreed with reports of other investigators. They concluded that fluorine exerts a direct local action upon the enamel-forming cells and that the changes evident in the enamel and dentine are not caused primarily by changes in blood calcium and phosphorus or by changes in the parathyroids.

Boddie (1945) reported that, although mottling of the enamel of the permanent incisors might be characteristic of chronic fluorine poisoning he did not consider that it necessarily had any harmful effect upon sheep. He stated that dental caries were extremely rare in sheep and that there was no evidence that caries incidence was increased by fluorine. In another article, Boddie (1947) concluded that the ingestion of relatively small amounts of fluorine may cause only dental changes during the development of permanent teeth of domestic animals. Adult animals, having permanent teeth, may ingest fluorine without ill effect on the teeth. If the amount of fluorine ingested is sufficiently high, however, bone dystrophy may occur in cattle and sheep of any age.

Seddon (1945) reported on dental fluorosis occurring naturally in sheep. The sources of fluorine were water and minerals supplied to the sheep. Two bands of sheep were observed. The estimated daily average fluorine intake in one group was 49 mg., and 73 mg. in the other. The effects on teeth were not readily apparent until the animals were two years old. During the third and fourth years, marked irregularity was noted in the wear of the incisors. The second pair of teeth was first affected, resulting in mottled enamel and chalky appearance of the shaft of the teeth. Marked wear soon followed. Later, the first and then the third and fourth pairs of incisors became similarly affected. By the time the animals reached four years of age the incisors had the appearance of those of animals eight years old. The molars also exhibited excessive and irregular wear.

Hurine (1949) referred to the widespread use of the term "mottling" and called attention to the need for an exact definition of the word. He stated that, according to Dean, Gordon, Moulton, and others, mottled enamel was essentially normal in form and contour but showed some decrease in translucency which might or might not be associated with surface glaze, irregular pitting and simple or variegated pigmentation. He reported his findings regarding the occurrence of opaque enamel in the permanent teeth of 170 persons reared in a "non-endemic" area in New England and concluded that opaqueness of the "white spots" type is not a reliable sign of chronic dental fluorosis.

Sarnat and Schour (1941) reported that enamel hypoplasia in human teeth is a dental manifestation of a constitutional disturbance which affects the temporary and permanent teeth being formed during the disease period. A narrow zone of defective enamel indicated 60 non-luetic patients whose teeth showed enamel hypoplasia. In addition, they made gross studies of more than 300 extracted teeth that exhibited enamel hypoplasia. Detailed medical history, with particular attention to infancy and childhood periods, was obtained from each patient. The incidence of hypoplastic enamel ranged from 5 to 17 percent. In more than one-half of the cases, no etiological factors were identified. The statement was made that no evidence of fluorosis was found in the patients studied.

Dean (1936) reported that with the continuous use of water containing about 1 ppm fluorine, it is probable that the very mildest forms of mottled enamel may develop in the teeth of about 10 percent of the people using this water. In waters containing 1.7 to 1.8 ppm, the incidence of mottled enamel may be expected to rise to 40 to 50 percent, although the percentage distribution of severity would be largely of the "very mild" and "mild" types. At 2.5 ppm, an incidence of 75 to 80 percent might be expected, with possibly 20 to 25 percent of all cases falling into the "moderate" or severer type. A scattered few may show the "moderately severe" type. "At 4 ppm the incidence is, in general, in the neighborhood of 90%, and as a rule 35% or more of the children are generally classified as 'moderate' or worse. In concentrations of 6 ppm or higher an incidence of 100% is not unusual."

Purpose and Scope of Study

Tennessee workers in 1947 became aware of a fluorosis problem affecting livestock in the area of the main station (Blount County Farm) and in the area of the sub-station at Columbia (Middle Tennessee Agricultural Experiment Station). Factors considered in connection with the problem included the proximity of an aluminum smelting production plant near Blount Farm, and phosphate operations plants and high phosphate soils in the Columbia area.

A preliminary study was made of livestock belonging to the University of Tennessee at these two locations to determine the source and extent of fluorosis in the various species and to provide a feasible approach to the solution of the problems.

Obvious questions were: (1) Could measures be applied by the industrial plants to control the escape of fluorine? (2) If the industrial plants were successful in controlling the emission of fluorine, were there other causes of the problem? (3) If causes of fluorosis could not be brought under control, should the University and the farmers in the areas change their operations or sell their farms and re-locate operations outside of these areas? (4) Would it be possible to alleviate effects of fluorine on the livestock?

Forage and feed analyses for fluorine, and a survey of the teeth of animals in the areas were used in determining the scope of the problem.

Representative animals, based upon the effects of fluorosis as diagnosed by the animals' permanent teeth, were sacrificed and autopsied to determine specific effects upon bones and tissues by means of gross and microscopic studies and by chemical analyses.

A check of published materials and discussions of the problem with Wisconsin, Ohio and Michigan workers made it evident that available information gave many suggestions on the problem, but provided relatively few specific research data.

The work of Phillips, *et al.* (1934) and Huffman, *et al.* (1930) was planned to determine the effect of adding raw rock phosphate as part of the mineral supplement in a dairy concentrate mixture. Because of the practice of feeding a dairy cow large amounts of concentrates when she is in heavy milk and little when she is not milking, these cows received large amounts of fluorine when in heavy production and considerably less when they were not milking. Phillips (1954) calculated that the fluorine in their total rations for the various treatments was, on the average, approximately 82 ppm, 150 ppm, and 296 ppm for two lactation periods.

The relative toxicity of the fluorine from industrial processing plants, as compared to fluorine from raw rock phosphate or sodium fluoride, is of tremendous interest. Some investigators have postulated that the fluorine from industrial sources is much more toxic than that in raw rock phosphate, calcium fluoride, or sodium fluoride. Small animal experiments have shown wide variation, but the majority of the data indicate that fluorine from raw rock phosphate (per gram of fluorine) is about 50 to 65 percent as toxic as that from sodium fluoride. There is need of further study on the effects of feeding farm animals known levels of fluorine derived from phosphate rock for comparison with results of feeding sodium fluoride.

From consideration of the literature, preliminary survey of data from animals and forage in the Blount and Columbia areas, and personal communications with investigators of other areas, it was concluded that, although fluorine analysis of forage within close proximity of an industrial plant might go up to several thousand parts per million, the practical problem for farmers in these and similar areas was gaining a knowledge of the effects of chronic low fluorine levels, on the average under 100 ppm, in the total ration. These preliminary observations further indicated that this fluorosis presented different problems with cattle, work stock, sheep and swine.

The initial experiments, therefore, were designed to attack the fluorine problem by way of the following objectives:

1. To study the effects of continuously feeding various levels of fluorine above normal amounts to cattle and sheep.

2. To compare the relative effects of sodium fluoride and effects of fluorine ingested in forages from areas near industrial plants.

3. To study the areas and determine the extent and effects of increased amounts of fluorine in the Blount County and Columbia areas.

4. To try to find a material (alleviator) which could be fed to cattle, sheep or work stock to counteract the effects of abnormal amounts of ingested fluorine.

These studies were under controlled, individual barn feeding conditions which would provide a biological curve for the effects of fluorine ingested by cattle. It was realized that continuous barn conditions are more drastic on cattle than normal summer pasture and winter feeding conditions, and that all groups concerned would question the results of barn conditions compared to practical field conditions. Therefore, similar cattle were put on pasture which was shown by analysis to have a high fluorine content for the area. These pastures were in the vicinity of the aluminum smelting plant in Blount County.

Methods of Sampling and Analyzing

Sampling Procedures. The supplements used in the experiments with cattle and sheep were mixed by hand in the early period of the tests and later in a twin spiral electric mixer of 1,000-pound capacity. These mixtures consisted of ground corn and a protein supplement. Fluorine and/or alleviators were added to these mixtures as indicated in Tables 1, 16 and 27. The additives were pre-mixed for uniformity with a small portion of the supplement, then added routinely to the supply for each individual lot as feeds were mixed. Representative samples from each batch were taken to make a composite sample for fluorine analysis.

Samples of hay were taken at the time of feeding and composited for periodic analyses. During the digestion trials, feed samples, feed refusals, and daily collections of urine and feces were taken and the representative aliquots refrigerated (7 or 10 days) and composited for analysis at the end of the studies. The pasture samples were taken every two weeks or monthly from various places throughout the pasture to obtain a representative sample of the forage consumed. Samples were taken every 50 paces in the pasture field, which was covered by a definite procedure, depending upon the forage and the shape of the field, to obtain a representative sample for analysis.

The forage samples were placed in a jar containing a fixative (calcium oxide plus ammonium carbonate) to prevent loss of fluorine during the storage period previous to being dried, ground and analyzed.

When silage was fed, representative samples were weighed, then dried for 48 hours at a temperature of about 60° centigrade, for analysis.

The chemical analyses employed to determine the constituents or groups of constituents which were considered in this experiment were made by either conventional or adapted procedures.

Fluorine was determined in the various materials by a slight modification of the perchloric acid distillation and thorium nitrate titration method as described by Willard and Winter (1933). Details of these modifications varied with the samples, since the materials included feeds, urine, feces and bones. Fluorine quantities in urine, feeds and soft tissues are reported in ppm fluorine on the basis of the sample taken. Fluorine content of bones is reported in ppm in the bone ash (to convert to a fat-free basis multiply the fluorine content on an ash basis by approximately two-thirds). Fluorine analyses provided not only data on fluorine concentrations but also information on the animal's average daily retention of fluorine when the fluorine intake and the excretion were known.

Proximate analyses of feeds and feces were determined according to procedures of the Association of Official Agricultural Chemists (1950). These analyses included ash, calcium, phosphorus, moisture, crude protein, ether extract, crude fiber and nitrogen-free extract, and provided the basis for the digestibility determinations.

Routine bone samples were taken for fluorine analysis from the right mandible, right metacarpal and right ninth and tenth ribs when an animal was autopsied. The mandible sample was taken from the angle of the right mandible. The metacarpal sample was taken from the medial proximal quarter of right metacarpal. The rib samples were obtained from the distal three to four inches of the ninth and tenth ribs. The rib biopsy samples were obtained from either the left ninth, tenth, eleventh or twelfth ribs.

Blood samples were taken periodically, from which serum was used for determinations of calcium, inorganic phosphorus, sodium, potassium, and magnesium. Calcium was determined according to the Clark-Collip modification of the Kramer-Tisdall method as described by Hawk *et al.* (1949).

The method of Fiske and Subbarow as described by Hawk *et al.* (1949) was used with slight modification, for the determination of serum inorganic phosphorus. Sodium and potassium were determined by use of a flame photometer as described in the Perkin-Elmer Instructions Manual (1949); and serum magnesium was determined by a combination of the method described by Simorsen *et al.* (1947) and the modified Denis method as described by Hawk *et al.* (1949).

Citrated whole blood samples were employed for the measurement of hemoglobin, packed cell volume, and specific gravity and for red and white blood cell counts. Additional fresh blood samples were taken for differential white cell counts. The procedures employed were as follows: hemoglobin, as described in the Evelyn Photoelectric Colorimeter Manual (1948); specific gravity, as described by Hawk *et al.* (1949), packed cell volume by centrifugation at 3,000 revolutions per minute for 15 minutes; white blood cell, red blood cell, and differential counts were made according to the procedure described by Parker (1948).

Beef Cattle Experiments

EXPERIMENT I, LOTS 1–11 AND 14–16

Objectives

The purpose of this experiment was to study the physiological changes in beef cows, associated with: (1) the feeding of various levels (0–100 ppm F) of fluorine as sodium fluoride, (2) the feeding of hays with different degrees of fluorine contamination, (3) pasturing on grass of different degrees of fluorine contamination, and (4) the feeding of high levels of calcium and phosphorus with fluorine.

The several phases of the experiment include effects of fluorine on:

1. Feed consumption and efficiency.
2. Growth, gains, and general physical condition.
3. Reproduction of cows and calf production.
4. Digestion and mineral balance.
5. Bones, including amount of fluorine stored in bones of cows and calves.
6. Various components of blood.
7. Urinary fluorine.
8. Teeth, from which studies, a method of classification of incisor teeth would be developed.

Experimental Procedure

The cattle used in this experiment were grade Hereford heifers purchased as yearlings in the Midwest in the winter and spring of 1948. The heifers were free from tuberculosis and Bangs disease when shipped. When they arrived at the Tennessee Station they were vaccinated with Prophylactic Brucella vaccine.

The first group of heifers was divided into 11 uniform lots of two animals each, based on source, weight, type, grade and condition. In addition to Lots 1 through 11, referred to as the barn-fed groups, 12 heifers were allotted to each of Lots 14 and 15, and 10 animals to Lot 16, to be designated as pasture groups. The heifers in Lots 1 through 11 were started on test in April, 1948. The second group of heifers in Lots 14, 15 and 16; and one additional animal each in Lots 1 through 11, were started in May and June, 1948.

Barn-Fed Lots. The heifers in Lots 1 through 11 were individually fed rations consisting of three parts ground No. 2 yellow corn and one part of 41 percent cottonseed meal. The cows were fed two pounds of concentrates from the beginning of experiment to November, 1948, three pounds of concentrates were fed from November, 1948, to February, 1949, and four pound for the remainder of period reported.

The fluorine was added to the concentrate mixture as sodium fluoride (NaF) in Lots 2 through 8 and 11. The amount of NaF added to the ration for each group was calculated on the ppm basis of the total air-dry ration consumed. At the end of each 28-day period the consumption was checked and the amount of NaF was adjusted according to the average daily consumption for each lot.

TABLE 1.—PLAN OF EXPERIMENT I, LOTS 1–11 AND 14–16

Lot no.	Number cows	F added in ration ppm	Total F in ration ppm	Av. initial wt., lbs.	Av. daily Mg. F/Kg. body wt.[c]
1	3	0	7	489	0.163
2	3	10	17	496	0.401
3	2[a]	20	27	479	0.615
4	3	30	37	500	0.856
5	3	40	47	507	1.064
6	3	50	57	510	1.310
7	3	70	77	462	1.699
8	3	100	107	473	2.132
9	3	B_1 Hay[c]		480	0.561
10	3	B_2 Hay[c]		495	0.868
11	3	100 + 0.5% Defluorophos[d]	107 + Defluorophos[d]	490	2.394
14	5[b]	B_2 Pasture and Hay		453[b]	
15	6[b]	B_1 Pasture and Hay		491[b]	
16	6[b]	Control Pasture and Hay		523[b]	

[a] One 2-year-old heifer was lost in calving in 1949, leaving only two.
[b] Plans in pasture groups were to start with 10 to 12 heifers and sacrifice these periodically. This was done periodically, which left the numbers shown and accounts for the variation in initial weight. Dates cows were sacrificed are shown in Table 10.
[c] The average milligrams of fluorine per kilogram of body weight is the calculated amounts consumed from April and June, 1948, to October, 1952. However, Lots 9 and 10 had a higher content during the first two years as discussed later.
[d] Defluorophos guaranteed analysis are: Ca not less than 31.00%, P not less than 13.10%, F not more than 00.05%. Ingredients; Defluorinated Phosphate containing not more than 500 ppm F and not less than 65.60% B.P.L. These results reported by International Minerals and Chemical Corporation.

Defluorinated rock phosphate was added to the concentrate ration of Lot 11 at the rate of 0.5 percent of the total air-dry ration.

The amount of NaF calculated for each lot was weighed in the Animal Nutrition Laboratory and put into a bottle marked for each respective lot (2 through 8 and 11). The concentrate mixture including NaF for each lot was prepared bi-weekly.

Hay used in these tests was good quality lespedeza-grass or alfalfa-grass mixtures containing at least 50 percent legume. The control hay was obtained from outside the fluorine area and samples were taken for chemical analyses, where possible, before purchase and again as fed. Control hay was fed to Lots 1 through 8 and 11. The hay fed to Lots 9 and 10 was purchased in sufficient quantity and quality to feed the cattle until the next hay season. It came from areas where fluorine was being emitted. Lot 10 was fed control concentrate and B_2 hay which contained as high an amount of fluorine as any hay that could be purchased in the affected area. Hay fed Lot 9 (B_1 hay) was selected to be of similar quality and to contain a fluorine content about midway between the control hay and B_2 hay. These hays were likewise analyzed for fluorine before purchase and again as fed. In the early part of the study all hay was fed as it came from the bale, but later it was chopped for feeding. Cows were fed equal amounts of their respective concentrate mixture, but the hay was individually fed *ad lib.* Individuals within a lot were offered the same amount of hay, but the amount fed daily was two or three pounds over the highest consumption of any one animal within that lot.

The cows were put into the stanchions at four to five o'clock each afternoon. After all cows were in their stanchions the concentrate was poured into the feeder. The cattle remained in the stanchions until five to seven o'clock A.M. After the cows were turned into a dry-lot, any hay or concentrate remaining in an individual stanchion was weighed and recorded. This procedure was varied during the hot part of the summer so that the cattle were in the barn during the day and outside in a dry-lot during the night. When the cattle were not in the stanchions they were kept in a dry-lot with free access to water and salt.

During the first two years, the cows at calving time were handled similarly. During the second year, considerable trouble was encountered from navel illness, resulting in losses of calves. During the third and fourth calving periods, the cows and calves were put into a pasture paddock a few days at calving time. The cows then went back to the regular schedule, but the calves remained in the clean lot while the cows were in the barn. After this practice was started the calves were nursed twice a day until weaning time.

Pasture Lots. Lot 14 cows were grazed on Blount 2 (B_2) pasture during the summer and kept during the first two winters in a barn with an outside paved lot. B_2 pastures were selected on the basis of chemical analysis for fluorine of the area. The pasture forage was estimated to average approximately 40 to 60 ppm F on an air-dry basis during the grazing season. The range of fluorine analysis was estimated to be about the range of fluorine content believed present on a number of the farms in the higher fluorine area, although it was realized that a few farms and areas might run higher in fluorine. From information available this level was considered to be a practical level for this area. The area was selected to be in one of the two prevailing wind directions from the aluminum smelting plant. Chemical analyses showed that the forage in the direction chosen had a more consistent fluorine content than areas to the side of prevailing wind directions. B_2 hay was fed during the winter. Most of the time it was the same as that fed to the barn-fed group, Lot 10.

After the first two winters the cattle were left on the pastures throughout the year and during the winter were fed hay in feeders placed in the pastures.

A concentrate mixture similar to that fed to Lot 1, barn control group, was fed to all three pasture groups, Lots 14, 15 and 16, during the first two winters. The pasture groups received no concentrate mixture during the third winter.

Lot 15 cows were grazed on Blount 1 (B_1) pastures, under management similar to that of Lots 14 and 16. B_1 pastures were selected on the basis of chemical analysis for fluorine of the forage in the area, estimated to average approximately 20 to 40 ppm F on an air-dry basis. This was about half-way between the fluorine content of Lots 14 and 16 during the grazing season.

The B_1 hay fed to this group was, most of the time, from the same source as hay fed to Lot 9 in the barn-fed groups.

Lot 16 cows were grazed on a pasture located some distance from the aluminum smelting plant. The pasture was believed to be out of the area of possible contamination with fluorine from the aluminum smelting plant. Animals in Lot 16 were managed and fed in a way similar to the treatment of Lots 14 and 15.

Pasture cows were put on the pastures about April 10 and were taken off about the first of October in the first two years. During the last two winters, the cattle remained on pasture with access to hay.

Cattle in Lots 1 through 11 and 14 through 16 had similar management. They were weighed at 28-day intervals. In the early part of the test, teeth were examined and records made at three- to six-month intervals. This time lapse was decreased until monthly readings were made. Natural-color pictures of the teeth were taken early in the experiment. It was in the spring of 1951, however, that the picture-taking technique was perfected so that satisfactory pictures were obtained for reproduction. Colored pictures of the teeth were taken approximately three times per year.

All cows were bred to calve at two years of age. At birth the calves were weighed and tattooed, and iodine was put on the navels. A leg bone was taken for chemical analysis from any calf lost or born dead. Check weights were taken on the calves at 28-day intervals, and two-day weights were taken at weaning time. The calves were weaned at approximately six months of age. The first calf crop was wintered after weaning and sold in the early spring. The 1950–51 calves were sold for slaughter at weaning time, and bones were taken for chemical analyses. This also was true of the calves from cows in Lots 1 through 11 in 1952, but from Lots 14 through 16 only the steer calves were sold. Heifer calves were carried on for data on their ability to grow out.

Samples for chemical analysis were taken regularly. Every two weeks a pasture sample was taken in the manner described in the Methods and Procedures section of this bulletin. Composite monthly samples of hay and composite three-month samples of the concentrate mixture were taken. Defluorinated phosphate was sampled as each batch was purchased. Rib biopsies for chemical analysis were performed on cattle in Lots 1 through 11. Bone samples were taken from all animals, calves or cows, when slaughtered or autopsied. Metabolism trials were conducted on animals in Lots 1 through 11.

Results and Discussion

Feed Consumption. The results of the study of feed consumed by periods are given in Table 2 for Lots 1 through 11. In studying and analyzing the feed consumption records, weight gains and the calving records of the cows should be considered.

For the period from April 1 to October, 1948, there was no appreciable difference in feed consumption between any lots. In studying the period from October, 1948, to October, 1949 (6 to 18 months on test), Lots 7 (70 ppm added), 8 (100 ppm added), and 11 (100 ppm added plus defluorophos), compared to Lot 1 (control lot), showed that the average daily consumption of hay per animal had decreased 1.97 lbs., 3.17 lbs. and 3.10 lbs., respectively. These differences are statistically significant. The concentrate consumption was appreciably lower only in Lot 8, which had a decrease of 0.96 lbs. per head daily.

During the period of October, 1949, to October, 1950 (one and one-half to two and one-half years on test), the cows in Lots 8 and 11 continued to consume less hay per animal daily than the other lots, with Lot 8 consuming less concentrates per animal daily. Lot 7, although consuming 0.85 lbs. per animal per day less than the control lot, did consume slightly more than Lot 10 only for the period. The cows in Lots 5 (40 ppm added) and 6 (50 ppm added) consumed less feed than Lots 1, 2, 3, 4, 9 and 10 during the period of October, 1950, to October, 1951 (two and one-half to three and one-half years on test). Thus, the control cows, Lot 1, consumed an average of 19.28 lbs. per animal daily, which was more by the

Table 2.—Effects of Fluorine on Feed Consumption of Cows in Experiment I, Lots 1–11

Lot no.	Total F in ration ppm	April 1948 to Oct. 1948 Av. daily Hay lbs.	April 1948 to Oct. 1948 Av. daily Conc. lbs.	Oct. 1948 to Oct. 1949 Av. daily Hay lbs.	Oct. 1948 to Oct. 1949 Av. daily Conc. lbs.	Oct. 1949 to Oct. 1950 Av. daily Hay lbs.	Oct. 1949 to Oct. 1950 Av. daily Conc. lbs.	Oct. 1950 to Oct. 1951 Av. daily Hay lbs.	Oct. 1950 to Oct. 1951 Av. daily Conc. lbs.	Oct. 1951 to Oct. 1952 Av. daily Hay lbs.	Oct. 1951 to Oct. 1952 Av. daily Conc. lbs.	Oct. 1948 to Oct. 1952 Av. daily Hay lbs.	Oct. 1948 to Oct. 1952 Av. daily Conc. lbs.
1	7	11.81	2.00	13.02	3.50	14.91	3.99	19.28	3.98	18.60	3.98	15.96	3.66
2	17	12.01	2.00	13.27	3.55	14.78	4.00	19.34	3.97	19.00	3.99	16.11	3.67
3	27	11.49	2.00	13.18	3.51	14.67	4.00	18.16	3.90	18.77	3.99	15.72	3.66
4	37	12.13	1.98	13.02	3.49	14.81	4.00	18.75	3.98	18.33	3.96	15.79	3.65
5	47	12.15	2.00	12.86	3.46	14.76	4.00	17.35 [b]	3.98	16.50 [a]	3.96	15.02 [a]	3.64
6	57	11.87	2.00	12.70	3.45	14.32	3.99	17.17 [b]	3.96	16.12 [a]	3.89	14.74 [b]	3.62
7	77	12.03	2.00	11.05 [b]	3.31	14.06 [a]	3.99	16.44 [b]	3.96	15.66 [b]	3.96	14.06 [b]	3.60
8	107	11.65	2.00	9.85 [b]	2.54	12.81 [b]	3.49	14.21 [b]	3.84	11.40 [b]	3.76	12.01 [b]	3.25
9	B_1 Hay	11.78	2.00	12.40	3.49	14.22	4.00	18.67	3.98	18.89	3.99	15.60	3.66
10	B_2 Hay	12.17	2.00	12.73	3.48	13.89 [a]	4.00	18.36	3.95	17.20	3.97	15.18	3.64
11	107 + Def.	11.64	2.00	9.92 [b]	3.25	13.46 [b]	3.99	14.30 [b]	3.96	13.36 [b]	3.98	12.64 [b]	3.60

[a] Statistically significant at .05 level.
[b] Statistically significant at .01 level.

following amounts than: Lot 5, 1.93 lbs.; Lot 6, 2.11 lbs.; Lot 7, 2.84 lbs.; Lot 8, 5.07 lbs.; and Lot 11, 4.98 lbs. These differences are statistically significant. There was no appreciable difference in concentrate consumption.

For the period of October, 1951, to October, 1952 (three and one-half to four and one-half years on test), Lot 1 had an average daily hay consumption of 18.60 lbs. per animal, which was 2.10 lbs. more than Lot 5, 2.48 lbs. more than Lot 6, 2.94 lbs. more than Lot 7, 7.20 lbs. more than Lot 8, and 5.24 lbs. more than Lot 11. These differences are statistically significant. In a comparison of the average daily feed consumption for the period from the spring of 1948, to October, 1952 (four and one-half years on test), there was no significant difference between the feed consumption of Lots 1, 2, 3, 4, 9 and 10. Considering Lot 5 (47 ppm total), there was a decrease of about 2 lbs. per head daily for the third and fourth years, and an overall decrease of 0.93 lbs. of hay per animal daily for the total period reported. The decrease in average daily hay consumption for the total period (four and one-half years) of Lots 6, 7, 8 and 11 compared to Lot 1 (control) was 1.22 lbs., 1.90 lbs., 3.95 lbs., and 3.32 lbs., respectively. These differences are statistically significant at one percent level for Lots 6, 7, 8 and 11 and at five percent level for Lot 5.

Considering all the experiments at this Station, and especially this experiment and Experiment III, except for teeth effects, the results indicate that one of the first measurable differences between animals (a difference caused by increased levels of fluorine ingestion where the level and duration of ingestion were sufficient to cause an effect) was a reduction in feed consumption.

One should keep in mind that these cows were started on fluorine feeding when they were about 12 to 15 months old, bred as yearlings at a light weight to calve as two-year-olds, and were about five and one-half years old in the fall of 1952. Further, these cows were fed continuously in barn and dry-lot without any pasture or succulent feed during the period reported. A comparison of these cows with pasture cattle, considering also the knowledge of the long feeding period under barn and dry-lot conditions, plus breeding of these heifers as young, light-weight yearlings, reveals that these cows were subjected to rigorous conditions which should contribute to maximum effects of increased levels of fluorine ingestion.

Table 3 shows the feed consumption of Lots 14, 15, 16, 25 and 26. Lots 14, 15 and 16 were put on test pastures in May, 1948, while Lots 25 and 26 were put on test pastures with Lots 16 and 14, respectively, in August, 1950. A study of the feed consumption data shows that the cattle in Lots 14 and 26 consumed more hay than the control groups, Lots 16 and 25. This does not follow the differences in barn-fed groups in comparing lots receiving similar amounts of fluorine from NaF (Lots 1 through 6).

Table 3.—Feed Consumption for 1948–1952 and Fluorine Content of Pasture Grass and Hays Consumed by Pasture Groups in Experiment I and II, Lots 14 and 26, 15, 16 and 25

	May 1948 to Oct. 1948	Oct. 1948 to April 1949	April 1949 to Oct. 1949	Oct. 1949 to April 1950	April 1950 to Oct. 1950	Oct. 1950 to April 1951	April 1951 to Oct. 1951	Oct. 1951 to April 1952	April 1952 to Oct. 1952	Total May 1948 to Oct. 1952
	Lot 14					Lots 14 and 26[a]				
Av. F content grass ppm	45.0		42.7		33.1	70.3	47.6	43.5	33.8	44.9
Av. F content hay ppm		66.1		60.2		33.0		30.0		51.0
Av. daily hay		13.8		16.1						
Av. daily conc.		3.1		4.0						
Mg. F/Kg. body wt.		1.26		1.11						
	Lot 15									
Av. F content grass ppm	28.4		32.4		16.6	27.1	25.3	16.5	19.3	24.9
Av. F content hay ppm		59.8		38.3		31.0		15.0		40.0
Av. daily hay		13.8		16.3						
Av. daily conc.		3.1		4.0						
Mg. F/Kg. body wt.		1.40		.80						
	Lot 16					Lot 16 and 25[a]				
Av. F content grass ppm	11.7		11.1		6.5	15.5	8.8	16.0	8.0	10.6
Av. F content hay ppm		13.2		7.3		5.0		4.5		8.0
Av. daily hay		13.8		18.0						
Av. daily conc.		3.1		4.0						
Mg. F/Kg. body wt.		.294		.202						

[a] Lot 26 containing 9 heifers and Lot 25 containing 6 heifers were added to Lots 14 and 16, respectively, approximately 2 years after Experiment I was started. (See Experimental Procedure of Experiment II.)

In comparing Lot 15 cows with any other group on a milligram per kilogram or ppm in feed basis, one should keep in mind that Lot 15, during the first winter at approximately 22 to 27 months of age, received hay that contained 59.8 ppm F compared to 66.1 ppm F in hay of Lot 14.

On a ppm basis, Lot 14, during the major period of teeth development, probably was most comparable to Lot 5 in the barn-fed group, while Lot 15 probably was most comparable to Lot 4, being above it during the first year and below during the third year.

Weights and Gains. A study of the average daily gains in Table 4 for the period of April to October, 1948, shows that of the barn-fed groups, Lots 4, 5 and 9, gained slightly more than the other barn-fed groups with Lot 11 making less gain. Within the pasture groups for this period, Lot 14 on B_2 pasture (containing an average of 45 ppm fluorine) gained appreciably more than any other pasture or barn-fed group. Lot 15 gained least of the pasture groups. A probable explanation for this difference is that dogs, kept at houses near B_1 pasture, molested the cattle and kept them in a nervous condition during the first two grazing seasons. This is further verified by weights in October, 1952, which show that the cows in Lot 15 were slightly heavier and had made slightly more gain (38 lbs.), than control, Lot 16, cows.

In analyzing the effects of various levels of ingested fluorine on weights and gains as shown in Table 4 for the period of April, 1948, to November, 1952 (four and one-half years on test), consideration must be given to the number of calves born and raised by each cow and each group of cows. Differences in feeds or management also must be considered. Lot 8 definitely made less gain. Considering the number of calves raised, Lot 11 (receiving 100 ppm F plus defluorophos), made more gain, had a better production record, and consumed more feed than Lot 8. This shows an advantage from feeding the defluorinated phosphate. Lot 7, the October, 1952, weight of which was the second heaviest for any barn-fed lot, had next to the poorest production record and was definitely below the records of Lots 1, 2, 3, 4, 5, 6, 9, 14, 15, and 16, and somewhat below Lot 11, which received more total fluorine daily.

Data to October, 1952, indicate that there was no appreciable difference in weights or gains of Lots 1, 2, 3, 4, 9 and 10 and probably no difference between these lots and Lots 5 and 6.

In a comparison of the pasture groups, Lot 14, which received an average of 44.9 ppm F in pasture grass and 51 ppm F in hay fed, had a higher total gain and a higher average daily gain compared to Lot 15, receiving 25 ppm F in pasture and 40 ppm F in hay, or the control pasture, Lot 16, receiving 11 ppm F in pasture and 8 ppm in hay, for the four and one-half year period.

Lots 25 and 26 were pastured and fed with Lots 16 and 14, respectively,

TABLE 4.—EFFECTS OF FLUORINE ON WEIGHTS AND GAINS OF COWS IN EXPERIMENT I, LOTS 1–11 AND 14–16
(All Weights Are in Pounds)

Lot no.	Total F in ration ppm	Initial weight	Weight Oct. 1948	Av. daily gain initial to Oct. 1948	Weight Oct. 1949	Av. daily gain Oct. 1948 to Oct. 1949	Weight Oct. 1950	Av. daily gain Oct. 1949 to Oct. 1950	Weight Oct. 1951	Av. daily gain Oct. 1950 to Oct. 1951	Weight Oct. 1952	Av. daily gain Oct. 1951 to Oct. 1952	Av. daily gain initial to Oct. 1952	Av. gain of cows by number of calves raised 0	1	2	3	4	5
1	7	489	626	.76	765	.38	926	.44	1092	.46	1113	.06	.38				.40(2)	.34(1)	
2	17	496	635	.77	754	.32	872	.33	1033	.44	1110	.20	.37		47(1)			.32(2)	
3	27	479	608	.75	724	.32	924	.55	1025	.28	1178	.41	.43				.43(2)		
4	37	500	653	.85	730	.21	878	.40	1049	.47	1217	.45	.44				.44(2)	.44(1)	
5	47	507	660	.85	721	.17	914	.53	1032	.32	1151	.32	.39			.38(1)	.40(2)		
6	57	510	636	.70	762	.34	830	.18	978	.41	1091	.30	.35			.42(1)	.36(1)	.28(1)	
7	77	462	596	.74	717	.33	914	.54	1078	.45	1206	.34	.45		.52(2)		.31(1)		
8	107	473	601	.71	675	.20	827	.42	970	.39	1010	.11	.32	.32(1)	.32(2)				
9	B_1 Hay	480	630	.83	764	.36	930	.46	989	.16	1100	.30	.38			.40(1)		.35(1)	.38(1)
10	B_2 Hay	495	637	.79	798	.44	920	.34	1051	.36	1095	.12	.36			.29(1)		.40(2)	
11	107 + Def.	490	602	.62	679	.21	838	.44	931	.26	1015	.22	.32		.33(1)	.39(1)		.24(1)	
14	B_2 Pasture	453 [a]	682	1.24	932	.72	1049	.32	1067	.05	1119	.14	.41		.60(1)		.46(1)	.33(3)	
15	B_1 Pasture	491 [a]	618	.69	773	.44	894	.33	980	.24	1073	.25	.36			.21(1)	.40(2)	.38(3)	
16	Control Pasture	523 [a]	679	.85	899	.63	969	.19	1017	.13	1067	.14	.33				.32(2)	.34(4)	

[a] Heifers were killed from each lot within the first two years, which accounts for differences in initial weight of the remaining heifers compared to other lots.

from August, 1950. Lot 26 had the highest average daily gain with probably no significant difference. Thus, considering all pasture lots, Lots 14 and 26, 15, 16 and 25, there are no appreciable differences in weights or gains for the periods on test from May, 1948, to October, 1952.

Reproduction and Calf Records. In studying the calving records one should keep in mind the following factors: (1) All lots of heifers were bred while weighing an average under 600 pounds and at a younger age than is recommended as a general practice. It was realized that breeding under such conditions probably would contribute to the cows' having difficulties at first calving. These conditions were more severe than those under which most farm herds would be managed. (2) Lots 1 through 11 were handled under continuous barn and dry-lot conditions for the four and one-half year period reported. It is common knowledge that, in general, for growing and producing cattle, pastures provide better nutrition, health, sanitation and production conditions than do continuous, year-round barn and dry-lot conditions. Lots 14, 15 and 16 were in the barn for the first two winters and were on pasture for all except these two periods. (3) There were only three animals per group; no animals were culled for any reason, such as not calving, calving late, poor production, etc., contrary to what would be done under good farm conditions. The herd of grade cows at the University of Tennessee Tobacco Experimental Station (TES), where 60 Hereford heifers were purchased in a feeder calf sale to start an experimental herd, provides the following record which can be used for comparison with the barn-fed and pasture groups. The cows at TES were managed under very good environmental conditions. They were not bred until they were two years old, to calve as three-year-olds. To start the experiment, 48 of the 60 two-year-olds were selected for breeding. In 1947, each of the 48 cows (100 percent of the total) raised calves. In 1948, 43 cows (89.6 percent of the originally selected 48) raised calves. In 1949, for the 3rd calf crop, 42 cows (87.5 percent of the originally selected group) raised calves. In 1950, for the 4th calf crop, 40 cows (83.3 percent of the original group) raised calves. In 1951, the 5th calf crop, 27 cows (56.2 percent of the original cows) raised calves. Many producers cull cows as was done in this herd, or fail to cull cows and are not aware of their true calf production. Thus, in evaluating the records of the barn-fed groups, which were under more rigorous conditions than the above herd, and which underwent no culling, one should consider all these factors before defining the effect of increased fluorine ingestion on calving records. Table 5 shows that there probably was no appreciable difference between the number of calves born and number of calves raised in Lots 1, 2, 3, 4, 5, 6, 9, 10, 14, 15 and 16. This is further substantiated by results from Lots 20 through 26 as shown in Table 19, which represents a repetition of part of this test.

The cows in Lot 11 gave birth to a smaller percentage of calves, with

TABLE 5.—EFFECTS OF FLUORINE ON REPRODUCTION AND CALVES OF COWS IN EXPERIMENT I, LOTS 1–11 AND 14–16 [c]

			1949			1950			1951			1952			Total 1949–1952		
				Calves raised			Calves raised			Calves raised			Calves raised			Calves raised	
Lot no.	Total F in ration ppm	No. cows	No. cows calving	No.	Av. daily gain	No. cows calving	No.	Av. daily gain	No. cows calving	No.	Av. daily gain	No. cows calving	No.	Av. daily gain	No. cows calving	No.	Av. daily gain
1	7	3	2	2	1.40	3	3	1.51	2	2	1.28	3	3	1.58	10	10	1.46
2	17	3	2	2	1.74	3	3	1.25	2	2	1.63	2	2	1.54	9	9	1.51
3	27	2 [a]	2	2	1.56	1	1	1.58	2	1	1.52	1	0	—	6	4	1.56
4	37	3	3	3	1.59	3	3	1.55	2	2	1.52	2	1	1.99	10	9	1.60
5	47	3	3	2	1.62	2	1	1.55	2	2	1.63	1	1	1.63	8	6	1.61
6	57	3	2	2	1.52	3	3	1.80	3	3	1.46	1	1	1.73	9	9	1.62
7	77	3	2	1	1.33	2	1	1.41	1	0	—	0	0	—	5	2	1.37
8	107	3	1	1	1.34	1	0	—	0	0	—	0	0	—	2	1	1.34
9	B_1 Hay	3	2	2	1.56	2	1	1.46	3	4 [b]	1.55	3	3	1.18	10	10	1.43
10	B_2 Hay	3	3	2	1.58	2	2	1.44	3	2	1.31	2	2	1.44	10	8	1.44
11	107 + Def.	3	2	1	1.43	2	2	1.49	2	2	1.28	1	1	1.49	7	6	1.41
14	B_2 Pasture	5	3	3	1.83	5	5	1.50	4	4	1.67	4	4	1.56	16	16	1.65
15	B_1 Pasture	6	3	2	1.33	4	4	1.85	6	6	1.60	6	6	1.74	19	18	1.65
16	Control Pasture	6	6	6	1.72	6	6	1.64	4	4	1.67	6	6	1.68	22	22	1.69

[a] One animal died June, 1949.
[b] One cow had twins.
[c] All calf weights have been adjusted to a comparable basis for sex, age and years.

probably no difference in percentage of calves raised from those born, compared to the lots above. The feeding of defluorinated phosphate definitely improved the feeding conditions as shown by feed consumption and calving records, in a comparison of cows in Lot 11 with cows in Lot 8.

Lots 7 and 8, under the conditions of this experiment, have a very poor record regarding the number of calves born and the number of calves raised.

The calves from all pasture groups, on the average, made higher average daily gains than calves from barn-fed lots. In comparing the calving records and average daily gains, Lots 14 and 26, and Lots 16 and 25 should be grouped. When these groups are studied together there is no appreciable difference in calving records or average daily gains of calves, for the first period of May, 1948, to October, 1952, between Lots 14 and 26 grazing pastures and consuming feeds that averaged 44.9 to 51 ppm (Table 3), Lot 15, consuming pastures and feeds averaging 24.9 ppm to 40.0 ppm, and control Lots 16 and 25 consuming pastures and feeds averaging 10.6 to 8 ppm.

Digestion and Balance Studies. In order to study the effects of various levels of fluorine ingestion by cows upon the various nutrients—crude protein, ether extract, crude fiber, nitrogen free extract, fluorine, calcium, phosphorus and nitrogen—complete balance trials with these cows were conducted in 1951 and repeated in 1952. The animals were barn-fed, as shown in Experiment I, Lots 1 through 11. They have been maintained on the respective rations indicated since 1948.

Balance trials likewise were conducted with cows from Experiment II, Lots 20A through 24B, as previously described and noted in Tables 20 and 21. These animals had been barn-fed their respective rations since October, 1950. The specific objectives of the balance studies were: (1) To determine the effects of the various levels of ingested fluorine upon the digestion of ration nutrients. (2) To determine the excretion and absorption of calcium, phosphorus, fluorine and nitrogen, as affected by the feeding of various levels of fluorine. (3) To investigate the effects of an alleviator ($Al_2(SO_4)_3 \cdot 18\ H_2O$) on the excretion and absorption of calcium, phosphorus, fluorine and nitrogen.

In the balance phase of the fluorine investigations, the cattle were placed in metabolism units equipped for quantitative separate collection of urine and feces, as described by Hobbs *et al.* (1950). Following an adjustment period of approximately seven days, a collection period of 7 to 10 days was started. Daily aliquots of feed, feces and urine were combined as a composite sample for the entire collection period. Chemical analyses for fluorine, calcium and phosphorus were made through conventional procedures by the Chemistry Department of this Station. All other analyses were made in the Animal Husbandry Nutrition Laboratory.

Results of these studies are presented in Tables 6 and 7. Table 6 shows the average dry matter intake and the average digestibility of nutrients as determined in 1951 and 1952, respectively. The 1951–52 summary in Table 6 was obtained in 1952. In agreement with over-all feed consumption data, Lots 7, 8 and 11 showed a decrease in feed consumption.

TABLE 6.—EFFECTS OF FLUORINE ON DIGESTIBILITY OF NUTRIENTS FED COWS IN EXPERIMENT I, LOTS 1–11

				Apparent Digestibility				
Lot no.	Total F in ration ppm	No. animals	Dry matter intake gms.	Dry matter percent	Crude protein percent	Ether extract percent	Crude fiber percent	Nitrogen-free extract percent
				1951				
1	7	3	7141	54.0	58.3	44.0	36.2	67.1
2	17	3	7035	62.5	66.8	52.5	47.0	73.7
3	27	1	5888	50.4	59.2	29.4	23.5	66.1
4	37	3	6751	65.9	67.4	54.8	48.7	75.7
5	47	2	6300	61.2	63.9	51.4	46.0	72.8
6	57	3	5945	63.5	66.6	52.7	53.1	74.6
7	77	3	5475	69.0	65.4	49.1	41.9	73.9
8	107	3	4601	65.6	71.5	57.0	49.6	76.1
9	B_1 Hay	3	6611	59.8	62.5	55.7	45.5	69.5
10	B_2 Hay	3	5663	65.0	64.8	57.3	51.3	74.1
11	107 + Def.	3	5139	65.0	67.7	56.1	50.8	75.1
				1952				
1	7	3	7377	62.4	66.6	61.7	52.2	68.9
2	17	3	7763	64.7	67.7	65.1	57.0	70.6
3	27	2	7632	62.8	65.8	62.4	54.0	68.8
4	37	3	7490	63.9	64.8	64.3	53.8	71.8
5	47	2	7520	64.6	69.7	65.4	51.0	73.0
6	57	2	7035	65.4	67.2	65.6	55.6	72.8
7	77	3	6827	65.5	66.6	60.6	57.1	73.2
8	107	1	3927	67.6	74.5	69.8	48.3	75.7
9	B_1 Hay	3	8029	69.7	71.2	53.7	65.9	75.8
10	B_2 Hay	2	7580	64.5	57.2	59.9	64.3	69.4
11	107 + Def.	3	7017	68.2	67.7	64.7	62.1	75.7
				Summary 1951 and 1952				
1	7	6	7259	58.2	62.4	52.8	44.2	68.0
2	17	6	7399	63.6	67.2	58.8	52.0	72.2
3	27	3	7051	58.7	63.6	51.4	43.8	67.9
4	37	6	7120	64.9	66.1	59.6	51.2	73.8
5	47	4	6910	62.9	66.8	58.4	48.5	72.9
6	57	5	6381	64.3	66.8	57.9	54.1	73.9
7	77	6	6151	67.2	66.0	54.9	49.5	73.6
8	107	4	4432	66.1	72.2	60.2	49.3	76.0
9	B_1 Hay	6	7320	64.8	66.8	54.7	55.7	72.6
10	B_2 Hay	5	6430	64.8	61.8	58.3	56.5	72.2
11	107 + Def.	6	6078	66.6	67.6	60.4	56.4	75.4

There were some variations in the digestibility of ration nutrients. These differences, however, were not definitely correlated with the level of fluorine added to the rations. There was some indication that the digestibility values were inversely related to the feed consumption during the balance period.

TABLE 7.—EFFECTS OF FLUORINE ON AVERAGE DAILY BALANCE OF FLUORINE, CALCIUM, PHOSPHORUS AND NITROGEN OF COWS IN EXPERIMENT I, LOTS 1–11, 1951–52

Lot no.	Total F in ration ppm	No. animals	Urinary Values		Fecal mg. F	Daily Balance			
			F ppm [a]	Total Mg. F		F mg.	Ca gm.	P gm.	N gm.
					1951				
1	7	3	2.8	23.5	42.0	−20.4	−4.2	−3.3	111.9
2	17	3	9.7	86.0	61.0	6.0	10.6	1.4	125.0
3	27	1	16.5	94.9	98.3	44.9	2.3	−4.0	97.2
4	37	3	18.1	159.8	132.6	93.4	16.0	2.3	126.6
5	47	2	19.8	160.6	196.3	109.3	4.9	−.8	110.2
6	57	3	20.9	155.1	155.5	219.3	16.9	1.6	106.5
7	77	3	31.7	171.2	180.2	156.0	2.6	.4	100.4
8	107	3	29.0	179.3	150.8	276.5	6.1	2.2	93.3
9	B_1 Hay	3	8.7	61.8	70.9	−15.5	−8.5	−2.2	121.2
10	B_2 Hay	3	13.9	97.0	79.1	9.6	10.4	.2	98.5
11	107 + Def.	3	36.8	247.7	287.5	354.5	13.2	3.2	96.5
					1952				
1	7	3	3.0	86.2	20.0	−52	3.7	4.9	32.6
2	17	3	9.2	65.4	37.2	71	14.7	2.4	19.4
3	27	2	18.4	117.4	52.8	106	5.5	4.7	28.6
4	37	3	21.0	135.9	76.1	189	13.5	1.1	10.8
5	47	2	29.1	191.8	91.8	200	8.9	5.3	39.4
6	57	2	37.5	231.0	136.4	210	8.6	4.4	23.8
7	77	3	41.4	180.3	157.5	384	−.3	0.0	15.1
8	107	1	69.0	264.4	93.9	321	3.7	−36.7	20.0
9	B_1 Hay	3	8.2	58.6	41.0	−3	−3.3	.9	25.9
10	B_2 Hay	2	24.4	122.8	94.2	50	1.3	−1.1	14.9
11	107 + Def.	3	39.5	224.8	257.2	519	−1.5	−.3	21.6
					Summary 1951 and 1952				
1	7	6	2.9	54.8	31.0	−36.2	−.2	.8	72.2
2	17	6	9.4	75.7	49.1	38.5	12.6	1.9	72.2
3	27	3	17.8	109.9	68.0	50.3	4.4	1.8	51.5
4	37	6	19.6	147.8	104.4	141.2	14.8	1.7	68.7
5	47	4	24.4	176.2	144.0	154.6	6.9	2.2	74.8
6	57	5	27.5	185.5	147.9	215.6	13.6	2.7	73.4
7	77	6	36.6	175.8	168.8	270.0	1.2	.2	57.8
8	107	4	39.0	200.6	136.6	287.6	5.5	−7.5	75.0
9	B_1 Hay	6	8.4	60.2	55.9	−9.2	−5.9	−.6	73.6
10	B_2 Hay	5	18.1	107.3	85.1	25.8	6.8	−.3	65.1
11	107 + Def.	6	38.2	236.2	281.4	436.8	5.8	1.4	49.0

[a] ppm fluorine corrected in urine to a specific gravity of 1.040.

Table 7 presents a summary of the balance studies for fluorine, calcium, phosphorus and nitrogen for the 1951 and 1952 periods, respectively. The fluorine content of the urine was corrected to a specific gravity of 1.040 in an attempt to compensate for the differences in the total daily amounts of urine voided. In most cases, the specific gravity of the urine was greater than 1.040. This probably was due to reduction of water intake and urine excretion which usually was observed when the cattle were placed in the metabolism stalls. It is noted that with increased fluorine intake there was an increase in the total excretion of fluorine in the urine and feces, and also an increase in the retention or total storage of fluorine. In general, about one-half of the fluorine excreted appeared in the feces, with the other half in the urine. The balance of fluorine was negative for some of the cows on the control ration, with no fluorine added, in both the 1951 and 1952 periods as shown in Tables 9 and 10. The exact cause of this variation, resulting in a negative balance, is not known. However, with the small amounts being consumed, the variation in chemical analyses and sampling may have contributed to the differences. Other possible explanations include: (1) The reduction in feed intake occurring when these cattle were placed in the metabolism stalls would give a corresponding decrease in the fluorine intake which may not have affected the endogenous excretion of stored fluorine. (2) The fluorine analyses for the feeds were consistently lower than the actual intake of fluorine. (3) The analyses of fecal and urinary fluorine may have been higher than the amounts actually excreted. Weighing and drying procedures for feeds or excreta may have accounted for some of this apparent error.

The average balances of calcium, phosphorus, and nitrogen are listed in Table 7. Difference in the balance of these elements were noted. However, these differences did not appear to be associated with level of fluorine added to the ration except possibly in the case of phosphorus at the higher levels.

Fluorine Content of Bones. Table 8 gives the data on cows that were sacrificed or which died. The plan was to kill cows from Lots 14, 15, and 16 to gain information on the uptake of fluorine in the bones. A comparison of an average fluorine content of leg and jaw bones of two cows in Lot 16 with three cows in Lot 14 showed that after 13 months on test Lot 14 had about 5.8 times as much fluorine. A similar comparison between Lots 16 and 15 shows that the cows in Lot 15 had about 3.6 times as much fluorine as the cows in Lot 16. The ratio was similar after 15 months on test. A comparison of the fluorine content of the bones of cows at 13, 18, 26 and 33 months on test showed that the fluorine level continued to go up.

A study of the data indicates that a relatively constant, continued level of fluorine fed in the ration will, in the earlier stages, cause the jaw bone to show a higher fluorine content than the leg bone.

The data from rib biopsies reported in Table 9 show that there is a direct relationship between the amount of fluorine ingested from the same source and the F content of the ribs. Lot 9, compared on a mg./kg. body weight basis, is similar to Lot 3. Table 9 shows that the fluorine content of ribs in Lot 9 was lower than the fluorine content of ribs of Lot 2. Likewise, the fluorine content of the ribs of Lot 10 (although comparable on a mg./kg. body weight basis with Lots 4 and 5) was lower than the rib content of Lot 3. These facts further confirm the data on teeth effects, which indicate that the fluorine on a ppm basis in forage from the area of an aluminum

TABLE 8.—EFFECTS OF FLUORINE ON BONE FLOURINE CONTENT OF ANIMALS SACRIFICED IN EXPERIMENT I, LOTS 14–16

Lot no.	Months on test [a]	F content in metacarpal ppm	F content in mandible ppm	Av. F analysis of pasture and hay for different periods: Pasture ppm	Hay ppm
14	13	4400	6100		
(B_2		4900	5900	54	66 [b]
Pasture)		5100	4100		
Av.		**4800**	**5367**		
	18	3900	5500		
		5000	6200	45	66 [b]
Av.		**4450**	**5850**		
	26	6600	7000	42	63
	33	7800	7200	45	60
15	13	3100	3300		
(B_1		2900	3100	34	60 [b]
Pasture)		3100			
Av.		**3033**	**3200**		
	15	2200	2800	36	60 [b]
	18	2100	4000		
		2200	3600	32	60 [b]
Av.		**2150**	**3800**		
16	13	700	1000		
(Control		800		14	13 [b]
Pasture)					
Av.		**750**	**1000**		
	18	730	890		
		840	900	12	13 [b]
Av.		**785**	**895**		

[a] From initial date until time of slaughter.
[b] Only one winter feeding period is represented in the respective lots.

TABLE 9.—EFFECTS OF FLUORINE ON RIB FLUORINE CONTENT OF COWS IN EXPERIMENT I, LOTS 1–11

Lot no.	Total F in ration ppm	Comparison of F in ribs from same animal: 1950 ppm	1953 ppm	Av. F content in ribs of animals in each lot—1953 [a] ppm
1	7	1500	1700	1300
2	17	3200	3500	3367
3	27	6200	5500	5300 [b]
4	37	7100	8200	6700
5	47	8900	8600	8200
6	57	10400	9000	9267
7	77	—	—	11567
8	107	12100	12700	12267
9	B_1 Hay	—	—	2367
10	B_2 Hay	—	—	4133 [b]
11	107 + Def.	12500	14200	13700

[a] Three animals represented in each average.
[b] Only two animals.

TABLE 10.—EFFECTS OF FLUORINE ON BONE FLUORINE CONTENT OF CALVES FROM COWS IN EXPERIMENT I, LOTS 1–11 AND 14–16

Lot no.	Total F in ration ppm	1950 Av. F content			1951 Av. F content		1952 Av. F content	Three Year Av. Av. F content	Average age in days
		Rib ppm	Mandible ppm	Metacarpal ppm	Mandible ppm	Metacarpal ppm	Metacarpal ppm	Metacarpal ppm	
1	7	325(2) [a]	230(2)	240(2)	125(2)	170(2)	90(3)	156(7)	164
2	17	347(3)	200(3)	243(3)	150(2)	135(2)	65(2)	161(7)	176
3	27	440(1)	300(1)	220(1)	100(1)	70(1)	220(1)	170(3)	114 [b]
4	37	210(3)	167(3)	240(3)	165(2)	150(2)	370(1)	232(6)	168
5	47	410(1)	240(1)	300(1)	145(2)	255(2)	140(1)	238(4)	186
6	57	275(2)	245(2)	320(2)	167(3)	247(3)	140(1)	253(6)	193
7	77	300(1)	250(1)	400(1)	—	—	—	400(1)	160
8	107	—	—	—	—	—	—	—	—
9	B_1 Hay	270(1)	190(1)	230(1)	160(4)	118(4)	103(3)	126(8)	166
10	B_2 Hay	695(2)	265(2)	335(2)	173(3)	240(3)	140(2)	238(7)	169
11	107 + Def.	450(1)	240(1)	320(1)	135(2)	215(2)	230(1)	245(4)	186
14	B_2 Pasture	2100(5)	1820(5)	1580(5)	1808(13)	1312(12)	813(3)	1304(20)	177
15	B_1 Pasture	748(4)	615(4)	655(4)	710(6)	542(6)	470(3)	560(13)	191
16	Control Pasture	777(6)	545(6)	607(6)	687(6)	425(6)	147(3)	442(15)	179

[a] The number in brackets gives the number of calves represented for the average F content reported.
[b] One calf in this group died when three days old.

TABLE 11.—FLUORINE CONTENT OF BONES FROM COWS AND THEIR FETUSES
(Cow Herd at Middle Tennessee Experiment Station, Killed in 1951)

Age	Right 9th rib ppm	Right 10th rib ppm	Right front meta-carpal ppm	Right rear meta-carpal ppm	Mandible ppm	Fetal bones: Rib ppm	Fetal bones: Meta-carpal ppm
			Cows, age 5–8 years, raised				
5	7200	7500	7400	7500	6400	620	330
6	7400	8200	8200	7800	7100	410	310
6	8800	8700	8400	8000	7000	380	310
8	8700	8700	11100	11300	5700	440	250
8	10500	10200	9800	9400	7700	470	270
8	9000	9200	9700	10000	8100	520	400
8	8900	8800	10300	9400	8500	360	230
8	8300	7600	9500	9300	6900	330	310
Av.	**8600**	**8612**	**9300**	**9088**	**7175**	**441**	**301**
			Cows, age 9–12 years, raised				
9	9200	9000	10100	9700	8500	280	270
9	9900	8900	10000	9800	9700	360	470
9	7900	7300	8800	8800	8200		
10	9400	9700	6400	9500	8600	330	210
10	11300	9200	10100	9000	8600	550	360
12	8600	8900	10000	10000	8400	270	190
12	9200	9600	11000	9700	8400	250	230
Av.	**9357**	**8943**	**9486**	**9500**	**8628**	**340**	**288**
			Cows, age 13–16 years, raised				
13			8500	9200	8000	260	220
13	8400	8100	8900	9300	7300	300	250
13	8100	9600	9500	9600	7500	300	290
14	8200	8400	8900	8900	8300	350	280
14	10000	9500	8850	8950	7600	360	300
16	8300	8400	8900	9300	8100	390	240
16	9900	10000	10700	10400	8300	350	240
Av.	**8817**	**9000**	**9178**	**9378**	**7871**	**330**	**260**
			Beef Heifers—Brought to Station in 1948				
4	7100	4900	6400	6200	7800		
4	7300	5700	6300	6000	5300	750	970
4	5100	6300	6000	5800	5200	440	610
4	6200	5700	6600	6000	6000	600	460
Av.	**6425**	**5650**	**6325**	**6000**	**6075**	**597**	**680**
			Cows and Heifers—Brought to Station in 1948				
8	5800	5100	5700	5500	5300	320	270
8	6300	6500	4300	4500	4700	390	370
6	5300	4300	4400	4400	4400	810	310
6	5000	4900	5200	4600	5800	450	350
5	4200	4700	4300	3800	4600		
4	5000	5600	5000	4400	5000	360	430
Av.	**5267**	**5183**	**4817**	**4533**	**4967**	**466**	**346**
Av.	**7887**	**7716**	**8102**	**8002**	**7094**	**414**	**336**

smelting plant resulted in less severe teeth effects and a lower bone storage compared to fluorine fed on ppm basis as NaF at the same rates.

The data presented in Table 10 reveal that the increased ingestion of fluorine by the cows in Lots 2 through 11 resulted in relatively little transfer of fluorine, to the fetus or through the milk, above amounts for the calves from Lot 1.

However, the bone fluorine data of calves from Lots 14 and 15, compared with Lot 16, showed that where calves are raised with cows grazing pastures with a high F content the calf bones have a higher F content because the calves grazed the pasture. Comparing the bone fluorine content of calves from Lots 14, 15 and 16 with Lots 1 through 11, the data showed that the calves apparently graze sufficient forage in the pasture to build up a higher F content in bones, depending upon F content of the pasture grass.

The data presented in Table 11 were obtained when 32 cows that had been on the Middle Tennessee Experiment Station were slaughtered in November, before they would have started calving in January. Twenty-nine fetuses were obtained from these cows for fluorine analysis. These cows may have ingested fluorine from industrial sources and high phosphatic soil. Studies are being continued in this area.

For comparison and study, data are presented by age groups, by groups raised or purchased, by different bones, and by each cow and the respective fetal bones. These data are on a bone ash basis, and would be about two-thirds as high on a fat-free basis.

The transfer of fluorine from cow to fetus apparently is not correlated with the fluorine content of the cow's bones. This further confirms the statement that increased fluorine ingestion by the cow, at levels studied in Experiment I, or in these data, does not materially increase the bone fluorine content of a fetus or nursing calf.

These cattle were not on feed with as high a level of fluorine in the season prior to slaughter as they had been previously.

Blood Studies. The function of blood as a carrier of nutrients and other materials including fluorine makes it of fundamental interest in this study.

Periodic blood samples were obtained by jugular stab from animals in the various experiments by the usual procedure (AOAC) and determinations were made of hemoglobin content, red and white blood cell counts, differential and cell pack volumes. Plasma specific gravity was measured by the method of Hawk *et al.* (1949) and calcium, phosphorus and magnesium were determined as outlined in the general experimental methods herein described.

The results of these analyses, expressed as lot averages, are presented in Table 12. The values are within the normal range as reported by Dukes (1947). Hematocrite, white and red cell counts, and plasma specific gravity did not appear to be significantly influenced by the dietary fluorine.

Table 12.—Effects of Fluorine on Blood from Cows in Experiment I, Lots 1–11 and 14–16

			Whole Blood					Differentials					Plasma	Serum		
Lot no.	Total F in ration ppm	No. animals	Hemoglobin (gms./100 ml.)	Hematocrit percent	Specific gravity	RBC (cmm.)	WBC (cmm.)	Eosinophiles percent	Basophiles percent	Neutrophiles percent	Lymphocytes percent	Monocytes percent	Specific gravity	Calcium (mgs./100 ml.)	Phosphorus (mgs./100 ml.)	Magnesium (mgs./100 ml.)
1	7	3	14.5	39.41	1.062	7,633,000	10,650	21	0	27	51	1	1.032	10.9	5.38	2.69
2	17	3	14.9	39.85	1.062	7,303,000	8,750	18	0	34	48	0	1.031	10.1	4.82	2.46 [b]
3	27	2	14.8	40.46	1.062	8,255,000	7,875	12	0	30	56	2	1.031	10.4	6.50	2.38
4	37	3	14.3	38.74	1.062	7,253,000	8,717	18	0	28	51	2	1.032	10.0	5.69	2.55
5	47	3	15.7	41.74	1.063	8,350,000	7,500	13	0	25	61	1	1.032	10.2	5.58	2.12
6	57	3	15.9	42.30	1.064	7,557,000	6,783	24	0	28	46	2	1.031	10.8	5.83	2.41
7	77	3	13.8	37.68	1.062	7,417,000	6,290	22	0	21	57	0	1.033	11.0	5.74	2.20
8	107	2	15.1	40.93	1.064	8,025,000	8,700	16	0	29	55	0	1.033	10.8	4.96	2.22
9	B_1 Hay	3	13.9	37.17	1.060	7,463,000	8,067	14	0	28	57	1	1.032	10.6	5.79	2.45
10	B_2 Hay	3	15.2	41.01	1.063	8,537,000	7,083	12	0	36	52	0	1.031	10.2	5.65	2.45
11	107 + Def.	3	14.0	38.39	1.062	7,203,000	8,317	21 [b]	0 [b]	24 [b]	54 [b]	0 [b]	1.032	10.9	5.14	2.36
14	B_2 Pasture	15	13.0	33.71	1.054	6,921,000	7,693	19	0	22	58	1	1.032	10.2	7.14	2.18
15	B_1 Pasture	6	13.5	34.20	1.059	6,668,000	7,467	11	0	35	53	1	1.032	10.6	4.71	2.43
16	Control Pasture	13	14.5 [a]	38.78 [a]	1.061 [a]	7,585,000 [a]	7,892 [a]	14 [a]	0 [a]	31 [a]	54 [a]	1 [a]	1.032 [a]	10.7	5.69	2.23

[a] Average of 12 animals.
[b] Average of 2 animals.

Neither were serum calcium, phosphorus and magnesium levels affected by the fluorine level in these rations. There was a wide variation in white blood cell differential counts, but this was not positively correlated with fluorine intake.

Urinary Fluorine. The possibility of using the fluorine content of the urine as an indication of the amount of fluorine being ingested has occurred to many workers. Blakemore *et al.* (1948) pointed out that the urinary level is influenced not only by the current rate of consumption and absorption from food, but also by the rate of excretion of the fluorine stored in the body from previous consumption. He also presented a method of standardizing urine samples to a uniform specific gravity to reduce the wide variation in the individual analyses.

The urine samples used in this study were collected from animals that were being held in digestibility studies previously discussed in this bulletin. The current rate of intake, as well as the dietary fluorine history of these cattle, was known. Samples for analysis were taken from single voidations, and from composites of one- and seven-day voidations. The results of these analyses, presented in Table 13, indicate that while there is a general trend

TABLE 13.—EFFECTS OF FLUORINE ON URINE FLUORINE CONTENT OF COWS IN EXPERIMENT I, LOTS 1–11

Lot no.	Total F in ration ppm	Single Voidation No. analyses	Single Voidation Av. F content ppm [a]	One-Day No. analyses	One-Day Av. F content ppm [a]	Seven-Day No. analyses	Seven-Day Av. F content ppm [a]
1	7	9	3.67	4	5.7	6	2.88
2	17	9	8.59	1	10.4	6	9.5
3	27	5	13.94	—	—	3	17.8
4	37	9	20.06	1	21.8	6	19.55
5	47	9	25.83	3	26.3	4	24.46
6	57	8	30.29	—	—	5	27.5
7	77	9	33.51	4	37.7	6	36.6
8	107	9	41.62	2	31.7	4	39.0
9	B_1 Hay	9	11.18	—	—	6	8.4
10	B_2 Hay	9	17.33	1	17.9	5	18.1
11	107 + Def.	9	54.13	4	41.1	6	38.1

[a] All values corrected to specific gravity of 1.040.

toward a correlation of the amount of fluorine excreted in the urine and the amount in the ration, this trend is of value only if there are several animals included in the analysis. This trend is the same whether the data represent single voidations or seven-day composites. Extreme caution must be used in cases where urine is available from only one or two animals. One of the highest uncorrected values in Lot 2, single voidation, was the same as the lowest uncorrected value for Lot 8; yet there is a difference of 90 ppm in the fluorine added to these rations. If the corrected values were used, the degree of overlapping was much less. Except for differences in ages and time on test, other feeding and management conditions were similar for Lots 1 through 11 and 20A through 24B.

While the data presented in Table 13 would indicate that there is some degree of relationship between the dietary intake and the urinary excretion, it is felt that the level of fluorine in the urine cannot be used as an absolute measure of the fluorine in the current ration. These data indicate that if a sufficient number of samples are taken and the average of these individual analyses is used, it may give a relative value for the fluorine being ingested. It is believed, however, that specific gravity of the samples of the urine should be measured in order that corrected values can be used for all calculations.

In trying to determine whether animals have fluorosis, urine should be used only with other factors such as; fluorine content of feeds and water consumed, a study of teeth of animals of various ages, fluorine content of bones of animals if possible, general condition of the herd along with a consideration of the feeding level and management conditions, and observations on each animal.

It appears from these data: (1) There is some relationship between the dietary intake and urinary excretion of fluorine. However, data herein indicate that the level of fluorine in the urine cannot be used as an absolute measure of the fluorine in the current ration. (2) If urinary excretion is used as one criterion for the estimation of the current dietary fluorine intake, a sufficient number of samples must be taken to allow for the wide variation in individual analyses. (3) The method of correcting urinary fluorine values to a urine of constant specific gravity reduces the range and variation of values found in uncorrected values.

Teeth. The importance of changes in teeth in the diagnosis of fluorosis in animals is recognized. Because of the emphasis that has been placed upon teeth changes as a measurement of the extent of structural and physiological effects associated with fluorosis, a comparative pictorial record of teeth from cattle consuming various levels of fluorine is presented. These teeth pictures were taken in the fall of 1952 (four and one-half years on test).

This is a summary of the effects on cattle teeth, pictures and descriptive details of teeth from animals in Experiment I. Immediately following this section, a summary of incisor teeth is presented in Table 15. The nomenclature and classification details for cattle teeth are presented following this section. The reader may wish to study these simultaneously with these pictures and detailed description. It is emphasized, however, that the described teeth effects represent only one of the many criteria employed in assessing overall effects of abnormal amounts of ingested fluorine.

Teeth pictures of animals in Lot 9 are not presented and only three animals from each of Lots 14, 15 and 16 because of expense.

DESCRIPTIONS AND PICTURES

LOT NO. 1 (7 ppm F)

ANIMAL NO. 42

CENTRALS: *Luster*—good; *Chalkiness*—focal, slight; * Longitudinal cracks; *Staining*—vegetative, slight; *Classification*—1A.

INTERMEDIATES: *Luster*—good; *Chalkiness*—focal, slight; * Longitudinal cracks; *Classfication*—1A.

LATERALS: *Luster*—good; *Chalkiness*—focal, slight,* milky plaque in lower ¼; *Staining*—vegetative, slight; *Classification*—1A.

CORNERS: *Luster*—good; *Chalkiness*—focal,* (right) lower ½ milky plaque, brown stained, (left) center ⅓ milky plaque, brown stained; *Classification*—1A.

GINGIVAE: Slight to moderate gingivitis.

PREMOLARS AND MOLARS: Normal stain and wear.

ANIMAL NO. 13

CENTRALS: *Chipped Cap*—(left) slight; *Luster*—good; *Chalkiness*— longitudinal, slight; * *Staining*—vegetative stain in grooves, slight; *Classification*—1A.

INTERMEDIATES: *Chalkiness*—focal, slight; *Staining*—vegetative; *Classification*—1A.

LATERALS: *Chipped Cap*—slight; *Chalkiness*—focal, slight; *Staining*—vegetative, slight; *Classification*—1A.

CORNERS: *Luster*—good; *Chalkiness*—focal; *Staining*—vegetative, slight; *Classification*—1A.

GINGIVAE: Slight to moderate gingivitis.

PREMOLARS AND MOLARS: Normal stain and wear.

ANIMAL NO. 24

CENTRALS: *Chipped Cap; Luster*—good; *Chalkiness*—vertical, cross, slight; *Staining*—vegetative, slight; root exposed 2 mm.; *Classification*—1A.

INTERMEDIATES: *Luster*—very good; *Chalkiness*—cross, slight; *Staining*—vegetative, slight; spacing—2.5 mm. between intermediates and centrals; *Classification*—1A.

LATERALS: *Luster*—good; *Chalkiness*—upper ¼ focal; *Staining*—vegetative; *Enamel hypoplasia*—transverse band which is located at junction of upper and middle thirds with loss of medial ⅕ of cap, pit. Hypoplastic abnormality is of unknown cause which makes this a 1-AX classification on hypoplasia alone, if due to fluorine it would be a 5-A classification (see discussion on enamel hypoplasia).

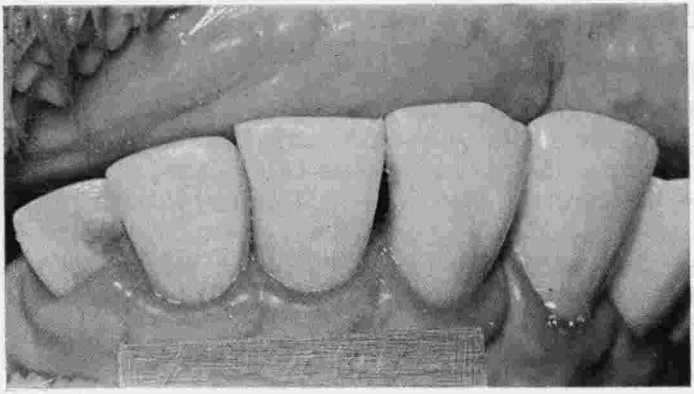
CORNERS: *Luster*—good; *Chalkiness*—focal, cross; *Staining*—vegetative, slight; *Classification*—1A.

GINGIVAE: Moderate to heavy gingivitis.

PREMOLARS AND MOLARS: Normal stain and wear.

Note the roughness of some enamel in incisor teeth of each mouth which is a common occurrence in normal teeth, especially in new teeth and on the corners.

* Not visible in pictures.

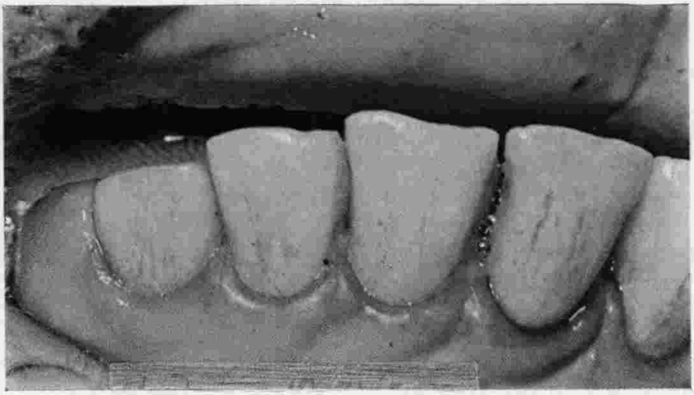


LOT NO. 1

Control Ration (7 ppm Fluorine)

Cow No. 42

Milky plaque in corner



Cow No. 13

Cow No. 24

Note: Chipped cap and uneven wear; roughness variation in all teeth; vegetative staining, especially No. 13; chalkiness, especially from centers to corners; enamel hypoplasia in laterals of No. 24; gingivitis in all animals

For details and discussion of normal teeth, terminology and details of teeth changes due to fluorine, reference is made to Section VI of this report.

An extensive study has indicated that such irregularities as chipped caps, slight chalkiness or mottling, vegetative staining, milky plaques, cracks, roughness and slight discoloration of enamel frequently are observed in teeth of apparently normal cattle. Occasional abnormalities such as the enamel hypoplasia noted in the lateral teeth of Cow No. 24 have been observed in apparently normal cattle. It is noted that this hypoplastic enamel condition, when compared with the enamel hypoplasia found in Lots 3–8, has not progressed as has the type of enamel hypoplasia characteristic of teeth of animals on the chronic fluorine levels.

All livestock consume small quantities of fluorine. If incisors are thoroughly cleaned and dried, slightly increased amounts of chalkiness or mottling may be observed in apparently normal teeth, reading progressively from the centers to intermediates, laterals and corners. Roughness of the enamel usually found in corner teeth of normal animals often makes it difficult to classify corner teeth of animals that have been affected with fluorine.

The observed gingivitis in the teeth of this control lot was found in general in all barn-fed lots, 1–11, but it was not observed in the pasture fed groups, Lots 14–16.

LOT NO. 2 (17 ppm F)

ANIMAL NO. 46

CENTRALS AND INTERMEDIATES: *Luster*—good; *Chalkiness*—focal, vertical striations, slight; *Staining*—vegetative, slight; *Classification*—1A.

LATERALS: *Chipped Cap*—very slight; *Luster*—good; *Chalkiness*—cross porcelain, medium; *Staining*—light brown, slight, vegetative; *Wear*—slight; *Classification*—2.

CORNERS: *Luster*—fair; *Chalkiness*—focal, cross and porcelain, medium, (left) milky plaque creamy; *Wear*—slight; *Classification*—1B.

GINGIVAE: Slight to medium gingivitis.

PREMOLARS AND MOLARS: FIRST, SECOND, FOURTH and FIFTH PAIRS: Normal. THIRD PAIRS: *Wear*—(uppers) slight. SIXTH PAIRS: *Wear*—(uppers) medium.

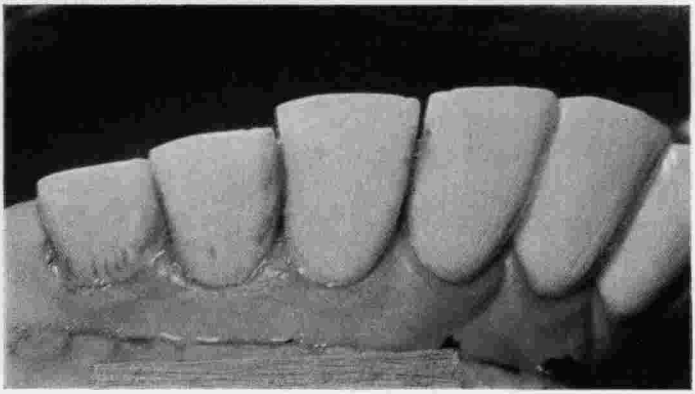
ANIMAL NO. 16

CENTRALS: *Luster*—fair; *Chalkiness*—focal, slight; *Staining*—vegetative, medium; *Wear*—uneven; *Classification*—1A.

INTERMEDIATES: *Luster*—fair; *Staining*—light brown, medium, lower ¾ vegetative; *Wear*—beveled, uneven; *Classification*—2.

LATERALS: *Chipped Cap*—slight; *Chalkiness*—focal, slight; *Staining*—brown, focal, medium, vegetative; *Classification*—2.

CORNERS: *Luster*—fair; *Chalkiness*—focal, heavy; *Staining*—light brown, vegetative, slight; *Enamel hypoplasia*—pit, slight; *Classification*—4.



LOT NO. 2

Control Ration + 10 ppm Fluorine added as NaF (Total F = 17 ppm)

Cow No. 46

Cow No. 16

Cow No. 11

GINGIVAE: Slight to medium gingivitis.

PREMOLARS AND MOLARS: FIRST, SECOND, FOURTH, and FIFTH PAIRS: Normal. THIRD PAIRS: *Staining*—light brown. SIXTH PAIRS: *Staining*—light brown; *Wear*—(uppers) medium to heavy.

ANIMAL NO. 11

CENTRALS: *Chipped Cap*—slight; *Luster*—fair to good; *Chalkiness*—focal, slight; *Staining*—light brown centrally, medium; *Classification*—2.

INTERMEDIATES: *Luster*—fair to poor; *Chalkiness*—focal; *Staining*—focal, brown, slight; *Caries*—superficial pre-carious foci; *Classification*—2, which may become 3 with time.

LATERALS: *Chalkiness*—(right) excessive, (left) focal; *Staining*—(right) brown, heavy, (left) yellow-brown, slight; *Caries*—(right) lower ½ (left) possible erosion, slight, black stains; *Enamel hypoplasia*—(right) suspicious; *Classification*—(right) 4, (left) 2 that may become 3.

CORNERS: *Chalkiness*—focal, diffuse; *Staining*—light brown, slight; *Enamel hypoplasia*—(right) pit, transverse, slight, (left) suspicious; *Classification*—5A.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST, SECOND, and FOURTH PAIRS: Normal. THIRD PAIRS: *Wear*—(uppers) slight. FIFTH PAIRS: *Wear*—(uppers) medium. SIXTH PAIRS: *Wear*—(uppers) excessive.

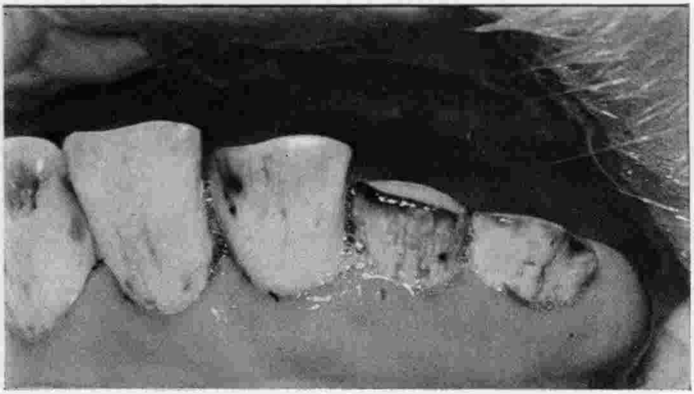
It should be noted, as a study is made of the pictures in Lots 1–11 and 14–16, that all cows were started on test about 12 to 15 months of age.

A comparison of the pictures of the center and intermediate teeth of all groups shows the immediate effects, upon developing teeth of varying levels of continuous fluorine ingestion.

A comparison of Lots 1 and 2 shows that the addition of 10 ppm F as NaF increased the amount and extent of chalkiness. Nos. 46 and 16 showed intraenamel stain on laterals, while No. 11 showed stain on centrals. Cow 46 exhibited no enamel hypoplasia whereas Cow 16 had enamel hypoplasia on corners and Cow 11 on laterals. Cow 11 exhibited erosion and caries on laterals, but none on any other teeth; nor were these defects exhibited by Cows 46 and 16.

Comparing the premolars and molars, the animals in Lot 2 showed some staining and wear on the third and sixth pairs. The wear apparently was first evident on the uppers. Cow 11 showed some wear on the uppers of the fifth pair. More staining and hypoplasia was shown on the incisors for Cow 11 than for Cows 46 and 16.

A study of the feed consumption, weights and gains, reproduction and calf records, digestibility of nutrients, and blood data showed no significant difference between Lots 1 and 2 under conditions of these tests. The rib biopsy fluorine data, urinary fluorine, and teeth effects, however, did show a direct relationship to the level of fluorine ingested.



LOT NO. 3

Control Ration + 20 ppm Fluorine added as NaF (Total F = 27 ppm)

Cow No. 32

Cow No. 47

(Note right and left sides)

Cow No. 47

LOT NO. 3 (27 ppm F)

ANIMAL NO. 32

CENTRALS: *Luster*—fair to good; *Chalkiness*—focal, slight to medium; *Staining*—(right) brown, focal, slight, (left) light yellow, slight; *Caries*—(right) upper ⅓; *Classification*—(left) 2, (right) 3.

INTERMEDIATES: *Luster*—good, upper ¼; *Chalkiness*—diffuse, lower ⅔; *Staining*—brown, slight to medium; *Caries*—(left) pre-carious pinhead, medially upper ½, (right) pre-carious upper ⅓, pinhead central lower ½; *Erosions*—(left) upper ½, (right) upper ⅓; *Wear*—slight; *Classification*—3.

LATERALS: *Chalkiness*—excessive; *Staining*—brown, heavy, black at cap; *Caries*—(left) pre-carious, pinhead lower ⅓ laterally; *Enamel hypoplasia*—slight; *Wear*—medium; *Classification*—4.

CORNERS: *Chalkiness*—excessive; *Staining*—focal, brown, slight to medium, transverse at cap; *Caries*—(left) pre-carious foci at cap; *Enamel hypoplasia*—slight; *Wear*—slight; *Classification*—4.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, slight. SECOND PAIRS: *Staining*—brown, slight; *Wear*—slight. THIRD PAIRS: *Staining*—brown, slight, *Wear*—medium. FOURTH PAIRS: Normal. FIFTH PAIRS: (lowers) normal, (uppers) long posteriorly. SIXTH PAIRS: *Staining*—brown, excessive *Wear*—(uppers) excessive, (lowers) long posteriorly.

ANIMAL NO. 47

CENTRALS: *Luster*—good; teeth spaced 1 to 3 mm.; *Classification*—1A.

INTERMEDIATES: *Luster*—upper ⅓ good; *Chalkiness*—upper ⅓ focal, cross, lower ⅔ diffuse chalky; *Staining*—transverse brown at junction of upper and central ⅓, light brown lower ⅔, heavy; *Wear*—slightly uneven; *Classification*—2.

LATERALS: *Luster*—fair; *Chalkiness*—focal upper ⅕, lower ⅘ diffuse; *Staining*—transverse upper ⅓, light brown, medium to heavy; *Caries*—(left) pre-carious lower ⅓, (right) pre-carious central ⅓; *Wear*—slight; *Classification*—3.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Erosions*—(right) medial ½ at cap; *Enamel hypoplasia*—slight; *Wear*—slight; *Classification*—4.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—slight. SECOND PAIRS: *Staining*—slight to medium, *Wear*—slight to normal. THIRD PAIRS: medium to heavy, *Wear*—medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—(lowers) brown, heavy, *Wear*—(uppers) excessive, (lowers) slightly long posteriorly.

A comparison of the teeth effects of cows in Lot 3 with those of cows in Lots 2 and 1 showed that the increase to 27 ppm F increased effects progressively as the teeth developed, particularly the amounts of chalkiness, intraenamel stain, caries and erosions, and enamel hypoplasia. The classification readings on the laterals and corners showed these progressive differences. The laterals and corners of this lot showed slight to medium wear above that for similar teeth in Lots 1 and 2. The first, second, third, and sixth pairs of premolars and molars showed staining. The second, third, and sixth pairs of premolars and molars showed some wear.

A comparison of the two pictures showing the right and left incisors of Cow 47 shows a close bilateral similarity. The definite line of demarcation in the intermediates shows the effect of increased fluorine ingestion on tooth development.

There was no appreciable difference between the feed consumption, weights and gains, reproduction and calf records, digestibility of nutrients, and blood data of cattle in Lot 3 compared to Lots 1 and 2.

The rib biopsy data, urinary fluorine and teeth effects, however, showed a direct relationship with the increased fluorine ingestion.

LOT NO. 4 (37 ppm F)

ANIMAL NO. 48

CENTRALS: *Luster*—upper ¼ good; *Chalkiness*—lower ½ diffuse, upper ½ cross and porcelain, heavy; *Staining*—light brown, medium to heavy; *Classification*—2.

INTERMEDIATES: *Luster*—medium at cap; *Chalkiness*—excessive; *Staining*—light brown, excessive; *Erosions*—(right) superficial, center near cap; *Enamel hypoplasia*—suspected; *Wear*—slight to medium; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light to dark brown, excessive; *Enamel hypoplasia*—slight to medium; *Wear*—medium; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—brown, excessive; *Enamel hypoplasia*—slight to medium; *Wear*—slight; (left) bucco-medioclination; *Classification*—5A.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) slight. THIRD PAIRS: *Staining*—brown, *Wear*—medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Wear*—(uppers) excessive, (lowers) shear anteriorly and long centrally.

ANIMAL NO. 30

CENTRALS: *Chipped Cap; Luster*—upper ⅓ good; *Chalkiness*—porcelain and cross, heavy, lower ⅓ excessive; *Staining*—light brown, heavy; *Erosions*—deep central ⅓, slight to medium; *Classification*—3.

LOT NO. 4

Control Ration + 30 ppm Fluorine added as NaF (Total F = 37 ppm)

Cow No. 48

Cow No. 30

Cow No. 31

INTERMEDIATES: *Chipped Cap; Luster*—good at cap; *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—pit, transverse lower ⅓, slight; *Wear*—uneven, slight; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—slight to medium; *Wear*—slight to medium; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell and patch, slight to medium; *Wear*—slight; *Classification*—5A.

GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—(uppers) brown, *Wear*—(uppers) medium to heavy, (lowers) slight. THIRD PAIRS: *Staining*—brown *Wear*—(uppers) heavy, (lowers) medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Wear*—(uppers) heavy to excessive, (lowers) shear anteriorly and long posteriorly.

ANIMAL NO. 31

CENTRALS: *Luster*—upper ⅓ good; *Chalkiness*—cross and porcelain upper ⅓, lower ⅔ excessive; *Staining*—brown, focal, medium; *Erosions*—superficial and undermining, dark brown centrally, medium; *Classification*—3.

INTERMEDIATES: *Luster*—upper ¼ fair; *Chalkiness*—excessive; *Staining*—light and dark brown, medium to heavy; *Caries* and *Erosions*—superficial and deep, medium; *Enamel hypoplasia*—slight; *Wear*—medium, (left) sheer; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, medium; *Tooth hypoplasia*—suspicious; *Wear*—medium; *Classification*—5B.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, medium; *Wear*—slight; *Classification*—5B.

GINGIVAE: Medium to heavy gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—(uppers) brown, *Wear*—(uppers) medium. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy, (lowers) medium to heavy. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Wear*—(uppers) excessive, (lowers) slightly long.

A comparison of the teeth effects of the cows in Lot 4 with those in Lots 1, 2 and 3 showed that the increased amount of fluorine resulted in a level sufficient to cause progressively greater effects with definite changes in the centrals. This comparison showed that the extent of teeth effects was directly related to the level of ingested fluorine and to the stage of tooth development when affected.

The effects on premolars and molars of cows in Lot 4, compared to Lots 1, 2, and 3, were progressively greater.

There was no appreciable difference between Lot 4 and Lots 1, 2, and 3 as shown by a comparison of the average daily feed consumption, weights and gains, reproduction and calf records, digestibility, and blood data.

The rib biopsy data, urinary fluorine and teeth effects revealed a direct relationship to increased fluorine ingestion.

LOT NO. 5 (47 ppm F)

ANIMAL NO. 49

CENTRALS: *Luster*—good to very good; *Chalkiness*—focal, cross; *Staining*—(left) pale yellow, focal in lateral ½, medium, slight labio-medioclination of left; *Classification*—(right) 1A, (left) 2.

INTERMEDIATES: This pair showed a non-bilateral effect which is observed occasionally. *Luster*—upper ¼ good, (right) good on lateral ¾ at cap; *Chalkiness*—(left) lower ¾ diffuse, (right) lower ⅘ excessive; *Staining*—(left) transverse yellow at junction of upper and central thirds and in lower ⅓, heavy, (right) excessive light brown to black in eroded areas; *Caries* and *Erosions*—(right) deep erosions upper ⅔, medium; *Enamel hypoplasia*—lower ⅓ slight; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Caries* and *Erosions*—(right) deep, undermining, heavy (left) undermining, slight; *Enamel hypoplasia*—(right) slight to medium and (left) slight; *Wear*—medium; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown to black, focal, excessive; *Caries* and *Erosions*—focal caries at cap, slight; *Enamel hypoplasia*—patch, medium; *Wear*—slight; *Classification*—5B.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) slight. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Staining*—brown, *Wear*—(uppers) slight. SIXTH PAIRS: *Staining*—excessive, *Wear*—(uppers) excessive and long posteriorly.

ANIMAL NO. 23

CENTRALS: *Chipped Cap; Luster*—upper ⅓ good; *Chalkiness*—upper ½ cross and porcelain, lower ½ diffuse; *Staining*—light brown to black, medium; *Caries* and *Erosions*—centrally slight; *Classification*—3.

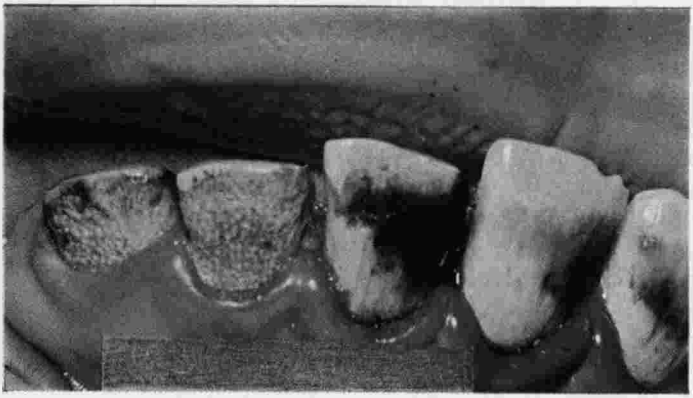
INTERMEDIATES: *Chalkiness*—longitudinal striations, excessive; *Staining*—black, heavy; *Erosions*—medium to heavy; *Enamel hypoplasia*—pit, slight; *Wear*—slight; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Caries* (right) focal; *Enamel hypoplasia*—patch, diffuse, medium; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5B.

LOT NO. 5

Control Ration + 40 ppm F added as NaF (Total F = 47 ppm)

Cow No. 49



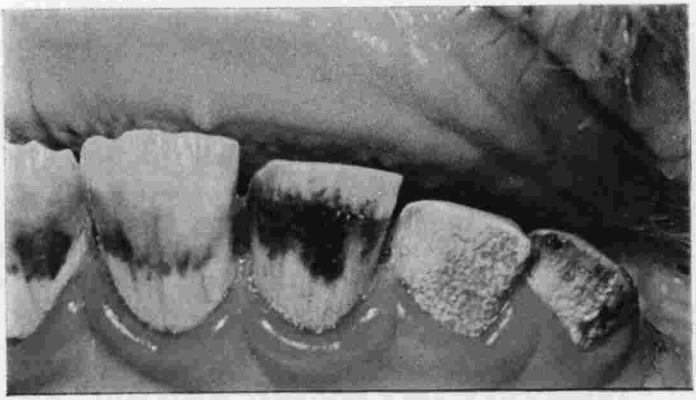
Cow No. 23

Cow No. 9

LOT NO. 5
(cont.)

Cow No. 49

Cow No. 23



Cow No. 9

CORNERS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Caries*—slight pre-carious foci and pinhead upper 1/3; *Enamel hypoplasia*—diffuse, patch, medium; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5B.

(This is a good example of bilateral effects on teeth.)

GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST and SECOND PAIRS: *Staining*—brown. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy and (lowers) slight. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—dark brown, excessive, *Wear*—(uppers) excessive and (lowers) long posteriorly.

ANIMAL NO. 9

CENTRALS: *Chipped Cap; Luster*—upper 1/5 good; *Chalkiness*—upper 1/4 cross and porcelain and lower 3/4 excessive; *Staining*—brown to black centrally, heavy; *Erosions*—superficial centrally, medium; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—(left) brown to black, excessive, (right) brown to black, heavy; *Caries* and *Erosions*—superficial and undermining, medium; *Enamel hypoplasia*—slight; *Wear*—slight to medium, (right) longitudinal cracks; Spacing—2 to 3 mm. from centrals; *Classification*—5A.

LATERALS: *Chalkiness*—excessive; *Staining*—(right) light brown, excessive, (left) brown to black, excessive; *Caries* and *Erosions*—(left) upper 1/4; *Enamel hypoplasia*—diffuse, patch, medium; *Tooth hypoplasia*—slight to medium; *Wear*—rolling, medium; *Classification*—5B.

CORNERS: *Chalkiness*—excessive; *Staining*—brown, excessive; *Enamel hypoplasia*—patch, medium; *Wear*—slight to medium; *Classification*—5B.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: Normal. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) medium. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy and (lowers) slight. FOURTH PAIRS: Normal. FIFTH PAIRS: *Staining*—(uppers) brown, *Wear*—medium, long posteriorly. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) excessive and (lowers) sheer anteriorly and long posteriorly.

MANDIBLE: This animal previously had lump jaw.

In general, the structural effects on the pairs of incisors appear to be bilateral and very similar. Inasmuch as members of a pair are usually in the same developmental stage, this is to be expected. However, individual members of a pair may not be similar. When one member of a pair is developed much earlier or later than the other member, the bilateral effect may be quite different. These facts were shown in this group, managed and fed in a similar manner, by a comparison of the intermediates of Cow 49 with intermediates of Cows 23 and 9. Differences were noted between the left and right intermediates of Cow 49.

The labio-medio-clination of the left central tooth of Cow 49 is an occurrence observed in some apparently normal teeth. The chipped caps in centrals of all cows in this lot, compared to the crown of the laterals and corners, demonstrate that a good tooth will chip while a fluorotic tooth will show a more crumbling effect.

A comparison of the teeth effects of cows in Lot 5 (47 ppm F) with those of Lots 1, 2, 3, and 4 revealed that the increased amount of fluorine ingested was reflected in progressive dental effects.

When the feed consumption of Lot 5 for the period of October, 1950, to October, 1951 (three and one-half years on test) was compared with the control lot, a statistically significant difference was shown. This small significant difference in feed consumption between Lot 5 and Lot 1 continued for the period reported. However, a study of the data showed no appreciable difference between weights and gains, reproduction and calf records, digestibility of nutrients, and blood data of Lot 5 compared to Lots 1–4.

The data for Lot 5 compared with those of Lots 1–4, showed that there was a direct relationship between the fluorine ingested and the fluorine content of the ribs, urine, and the teeth effects.

LOT NO. 6 (57 ppm F)

ANIMAL NO. 50

CENTRALS: *Chipped Cap; Luster*—good at cap; *Chalkiness*—focal, porcelain, cross with lower ⅓ diffuse; *Staining*—transverse at junction of lower and central ⅓ and brown-black in medial portion of central ⅓; *Erosion*—superficial in medial portion of central ⅓; exposed root; *Classification*—3.

INTERMEDIATES: *Chipped Cap; Luster*—fair; *Chalkiness*—focal at cap; lower ¾ diffuse, excessive; *Staining*—light yellow, slight; *Enamel hypoplasia*—pit, slight; *Wear*—uneven, slight; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, pit, slight to medium; *Wear*—medium; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, medium; *Wear*—slight to medium; *Classification*—5B.

GINGIVAE: Slight to medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—brown, *Wear*—medium. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy, (lowers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—(uppers) medium and long posteriorly. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) excessive, (lowers) excessive and uneven.

LOT NO. 6

Control Ration + 50 ppm Fluorine added as NaF (Total F = 57 ppm)

Cow No. 50

Cow No. 6

Cow No. 1

ANIMAL NO. 6

CENTRALS: *Chipped Cap; Luster*—good at cap; *Chalkiness*—focal, cross and porcelain at cap, lower 4/5 focal to excessive; *Staining*—light brown to brown, heavy; *Caries*—pre-carious foci lower 1/2, apparently non-hypoplastic pits in upper 2/3; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Caries* and *Erosions*—pinhead upper 1/3; *Enamel hypoplasia*—slight; *Wear*—slight to medium; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—focal dark brown, excessive; *Enamel hypoplasia*—pit, patch, slight to medium; *Wear*—slight to medium; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—light to dark brown, excessive; *Enamel hypoplasia*—patch, medium; *Wear*—slight; *Classification*—5B.

GINGIVAE: Slight to medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) medium. THIRD PAIRS: *Staining*—brown, *Wear*—medium to heavy. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—brown-black, *Wear*—(uppers) excessive, (lowers) sheer anteriorly and long posteriorly.

ANIMAL NO. 1

CENTRALS: *Chipped Cap; Luster*—upper 1/4 fair; *Chalkiness*—excessive; *Staining*—transverse, brown-black at junction of upper and center 1/3 and light brown lower 1/2; excessive; *Caries* and *Erosions*—transverse upper 1/3, slight to medium; *Enamel hypoplasia*—suspected; *Wear*—uneven, slight; *Classification*—4.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Caries*—slight; *Erosions*—upper 1/5, deep; *Enamel hypoplasia*—pit, patch, medium; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5B.

LATERALS: *Chalkiness*—excessive; *Staining*—very light brown, excessive; *Enamel hypoplasia*—shell, patch, medium; *Wear*—slight; *Classification*—5B.

CORNERS: *Chalkiness*—excessive; *Staining*—very light brown, excessive; *Enamel hypoplasia*—shell, patch, medium; *Wear*—slight; *Classification*—5B.

GINGIVAE: Slight to medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) slight. THIRD PAIRS: *Staining* brown-black, *Wear*—(uppers) slight. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—brown-black, *Wear*—(uppers) excessive (lowers) sheer anteriorly and long posteriorly.

A comparison of the teeth effects of cows in Lot 6 with those in Lots 1, 2, 3, and 4 showed that the increased amount of fluorine ingested is reflected in progressive teeth changes. However, a comparison with the teeth of Lot 5 (47 ppm F) showed that the increase of 10 ppm F at this level produced only a small change in the effects on teeth.

The intermediate teeth of Cow No. 1 showed slight tooth hypoplasia, as pointed out in the detailed teeth description. It is difficult to determine slight tooth hypoplasia because of the variation in tooth spacing in some animals, enamel hypoplasia, and the amount of wear in hypoplastic teeth.

A comparison of the feed consumption of Lot 6 for the period from April, 1948, to October, 1952, with the feed consumption of Lot 1 showed that there was a statistically significant difference for the years of October, 1950, to October, 1951, and from October, 1951, to October, 1952. These differences were progressive and showed a statistically significant difference for the total period.

A study of the data for Lot 6 showed that there was a direct relationship between the fluorine ingested and the fluorine content of ribs and the urine, and with the fluorine balance when compared to that for Lots 1–5 under conditions of this test.

There were no appreciable differences between weights and gains, reproduction and calf records, digestibility of nutrients, and blood data between Lot 6 and Lot 1.

LOT NO. 7 (77 ppm F)

ANIMAL NO. 2

CENTRALS: *Chipped Cap*—left; *Luster*—good upper ¼; *Chalkiness*—porcelain at cap, longitudinal, slight, lower ¾ excessive; *Staining*—brown and black, (right) excessive, (left) medium; *Caries*—(left) multiple pinpoint and pinhead; *Erosions*—(right) superficial, heavy, (left) slight; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive, focal black; *Caries* and *Erosions*—slight on medial margins; *Enamel hypoplasia*—patch, heavy; *Tooth hypoplasia*—slight; *Wear*—slight to medium; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, heavy; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, heavy; *Wear*—slight; *Classification*—5C.

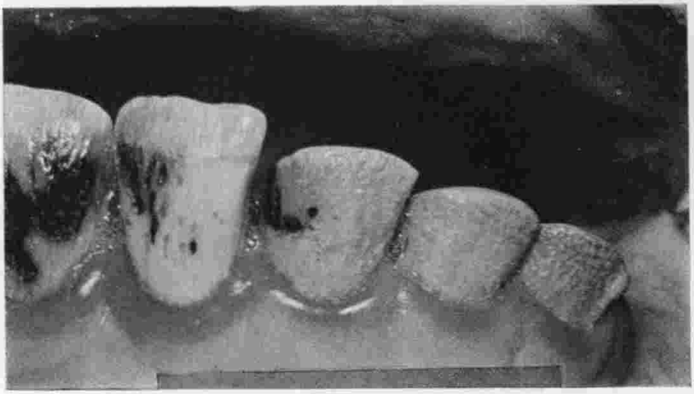
GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown-black. SECOND PAIRS: *Staining*—dark brown, excessive, *Wear*—(uppers) heavy and (lowers) slight. THIRD PAIRS: *Staining*—brown-black, excessive, *Wear*—(uppers) medium to heavy, (lowers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: (uppers) long posteriorly. SIXTH PAIRS: *Staining*—brown-black, excessive, *Wear*—(uppers) excessive to gum line and (lowers) long posteriorly and sheer anteriorly.

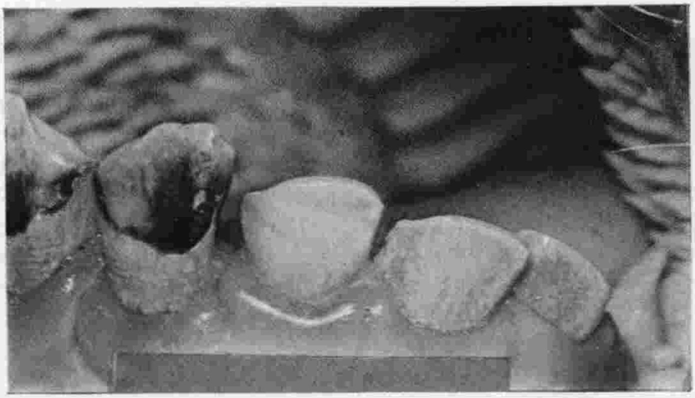
LOT NO. 7

Control Ration + 70 ppm
Fluorine added as NaF
(Total F = 77 ppm)

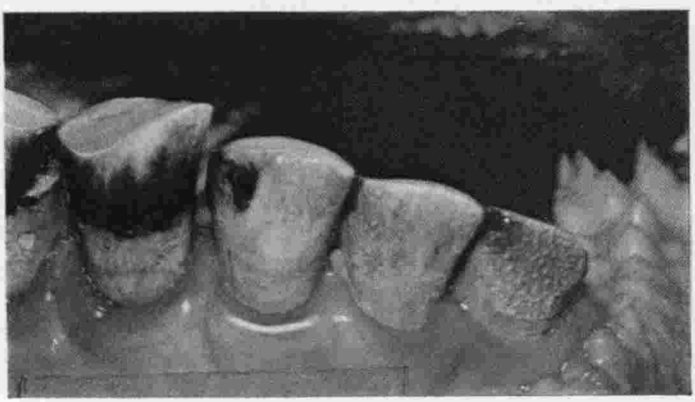
Cow No. 2



Cow No. 58



Cow No. 21



ANIMAL NO. 58

CENTRALS: *Luster*—fair, upper ⅛; *Chalkiness*—excessive; *Staining*—light and dark brown, excessive; *Erosions*—superficial and undermining, heavy to excessive; *Enamel hypoplasia*—patch, lower ⅓, slight; *Classification*—5A.

INTERMEDIATES AND LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell and focal, patch, heavy; *Tooth hypoplasia*—medium; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell and focal, patch, heavy; *Tooth hypoplasia*—medium; *Wear*—medium; slight bucco-labio-clination; *Classification*—5C.

GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) slight. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) slight. FOURTH PAIRS: Normal. FIFTH PAIRS: *Staining*—light brown, slight. SIXTH PAIRS: *Staining*—(uppers) brown, excessive, *Wear*—medium and (lowers) long posteriorly.

ANIMAL NO. 21

CENTRALS: *Luster*—fair lateral cap; *Chalkiness*—excessive; *Staining*—brown and black-brown, excessive; *Erosions*—superficial and undermining, heavy to excessive; *Enamel hypoplasia*—patch, lower ⅓ slight; *Classification*—5A.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive, focal black; *Erosions*—medially near cap, black stained, slight; *Enamel hypoplasia*—shell, patch, heavy; *Tooth hypoplasia*—slight; *Wear*—slight to medium; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell, patch, heavy; *Tooth hypoplasia*—slight; *Wear*—slight to medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive with dark foci at cap; *Enamel hypoplasia*—patch, heavy; *Wear*—slight; *Classification*—5C.

GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—dark brown, *Wear*—(uppers) slight. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) slight. FOURTH PAIRS: Normal. FIFTH PAIRS: (uppers) long posteriorly. SIXTH PAIRS: *Staining*—brown, black, *Wear*—(uppers) excessive and (lowers) heavy, uneven, long posteriorly.

The increase from 50 to 70 ppm of ingested fluorine as sodium fluoride resulted in marked changes, and the overall differences between these levels of feeding were greater than between any of the previous groups.

A comparison of the daily feed consumption between cows in Lot 7 and Lot 1 showed a highly significant difference by the end of one and one-half years on test (for the period of October, 1949, to October, 1950). The cows in Lot 7 were lighter in weight and lower in condition than those in Lots 1–6 during the first eighteen months. This lowered condition is thought to have caused the reduction in number of calves produced compared to Lots 1–6, 9 and 10. This decreased calf production for the period probably enabled Lot 7 to have the second highest body weight in October, 1952. A study of the rib biopsy data and teeth revealed progressive effects due to increased fluorine ingestion, compared to Lots 1–6. However, there was no apparent difference in the digestibility of nutrients or blood data in comparing Lot 7 with Lot 1.

Teeth effects of Lot 7 compared with Lots 1–6, showed a progressive increase in severity due to the increased level of fluorine ingestion. Since all lots were started on test at relatively the same age and time, the differences of teeth effects in Lot 7 compared to other groups were shown in the amount of tooth hypoplasia and the thin type of enamel on the intermediates, laterals, and corners. Likewise, a study of the classification showed that Lot 7 was the first lot having readings of 5 on any of the centrals, (Cows No. 58 and No. 21).

LOT NO. 8 (107 ppm F)

ANIMAL NO. 28

CENTRALS: *Chipped Cap; Luster*—fair at cap; *Chalkiness*—excessive, porcelain in cap area; *Staining*—light brown to black, excessive; *Erosion*—superficial, deep and undermining centrally, heavy; *Enamel hypoplasia*—patch, lower ¼ slight; *Wear*—slightly uneven; *Classification*—5A.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—very light brown, excessive; *Enamel hypoplasia*—patch, shell, heavy; *Tooth hypoplasia*—slight; *Wear*—slight; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—yellow to light brown, excessive; *Enamel hypoplasia*—shell, heavy; *Tooth hypoplasia*—slight; *Wear*—slight to medium; *Classification*—5C.

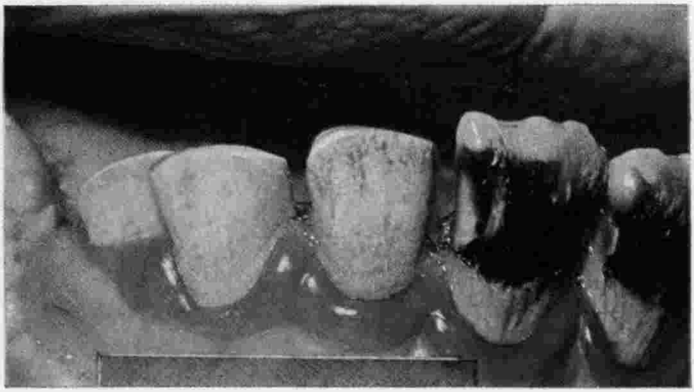
CORNERS: Similar to laterals except slight wear and slight bucco-medioclination; *Classification*—5C.

GINGIVAE: Medium gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) slight. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) medium to heavy and (lowers) slight to medium. FOURTH PAIRS: Normal. FIFTH PAIRS: (uppers) gouged posterior third with long spicule at extreme posterior. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) excessive and (lowers) medium, long centrally.

ANIMAL NO. 29

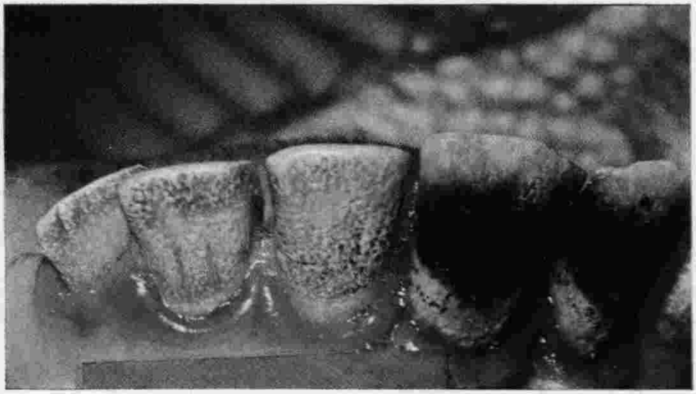
CENTRALS: *Chipped Cap; Luster*—fair at cap; *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Erosion*—superficial, deep, un-



LOT NO. 8

Control Ration + 100 ppm Fluorine added as NaF (Total F = 107 ppm)

Cow No. 28



Cow No. 29

Cow No. 61

dermining, heavy, centrally; *Enamel hypoplasia*—patch, lower ⅓ slight; *Classification*—5A.

INTERMEDIATES AND LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell, patch, heavy; *Wear*—slight; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell, patch, heavy; *Wear*—slight; medio-bucco-clination; *Classification*—5C.

GINGIVAE: Heavy gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—dark brown. SECOND and THIRD PAIRS: *Staining*—dark brown, *Wear*—(lowers) slight, (uppers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—(uppers) medium, long posteriorly. SIXTH PAIRS: *Staining*—(lowers) brown, *Wear*—(uppers) heavy, long posteriorly, (lowers) medium, long centrally with sharp medial points.

ANIMAL NO. 61

CENTRALS: *Chipped Cap; Luster*—fair at cap; *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Erosions*—superficial, deep and undermining centrally, heavy; *Enamel hypoplasia*—patch, lower ⅕ slight; *Classification*—5A.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Erosions*—deep, undermining centrally and medially, slight; *Enamel hypoplasia*—patch, shell, heavy; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Enamel hypoplasia*—patch, heavy; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Enamel hypoplasia*—patch, shell, heavy; *Tooth hypoplasia*—slight; *Wear*—medium; *Classification*—5C.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) slight. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—medium. FOURTH and FIFTH PAIRS: Normal, except (upper) 5th is long posteriorly. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) excessive, (lowers) medium, uneven, slightly long posteriorly.

Teeth effects due to the increased level of ingested fluorine in Lot 8, compared with Lots 1–6, showed results similar to the comparison to Lot 7, except that the effects were slightly more severe. This was observed in comparing the centrals and 1st and 5th molars on the basis of all animals within a group.

The average daily feed consumption of Lot 8, compared to Lot 1, was lower for any period considered and was statistically significant for the period of October, 1948, to October, 1949; and for the period from April,

LOT NO. 10

B_2 Hay + Control Concentrate

Cow No. 19

Cow No. 67

Cow No. 20

1948, to October, 1952. Lot 8 definitely had lower weights and gains than Lots 1–7, and a lower record of reproduction and calf performance, including overall production.

The lower record of calf production was deemed to be due to the low level of nutrition. The lower nutritional level was attributed to the higher level of fluorine ingestion, in comparing Lot 8 to Lots 1–6. As the cows are sacrificed, detailed studies will be made as to possible causes of the lowered reproduction.

LOT NO. 10 (B_2 Hay)

ANIMAL NO. 19

CENTRALS: *Chipped Cap; Luster*—upper ⅓ good; *Chalkiness*—porcelain, cross upper ⅓, lower ⅔ excessive; *Staining*—very light brown centrally, slight; *Wear*—normal, uneven; *Classification*—2.

INTERMEDIATES: *Luster*—fair; *Chalkiness*—heavy to excessive; *Staining*—very light brown, excessive; *Caries*—(left) upper ⅓ pinpoint, superficial; *Enamel hypoplasia*—suspected; *Wear*—(left) medium, (right) slight; *Classification*—4.

LATERALS: *Chalkiness*—diffuse; *Staining*—light brown, slight to medium; *Classification*—2.

CORNERS: *Chalkiness*—cross and porcelain at cap, lower ⅘ diffuse; *Staining*—light brown, heavy; *Classification*—2.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—light brown. SECOND PAIRS: *Staining*—light brown, *Wear*—(uppers) medium, (lowers) slight. THIRD PAIRS: *Staining*—light brown, *Wear*—slight. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—slight. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) heavy, (lowers) medium, long posteriorly.

ANIMAL NO. 67

CENTRALS: *Luster*—good; *Chalkiness*—focal, cross upper ½; *Staining*—vegetative; *Classification*—1A.

INTERMEDIATES: *Luster*—fair at cap; *Chalkiness*—upper ⅕ porcelain, and cross and lower ⅘ diffuse chalky; *Staining*—focal, diffuse; *Enamel hypoplasia*—pit, suspected to slight; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—diffuse, pit, slight; *Wear*—slight; *Classification*—5A.

CORNERS: *Chalkiness*—excessive; *Staining*—medium brown, excessive; *Enamel hypoplasia*—diffuse, pit, slight to medium; *Wear*—slight; *Classification*—5A.

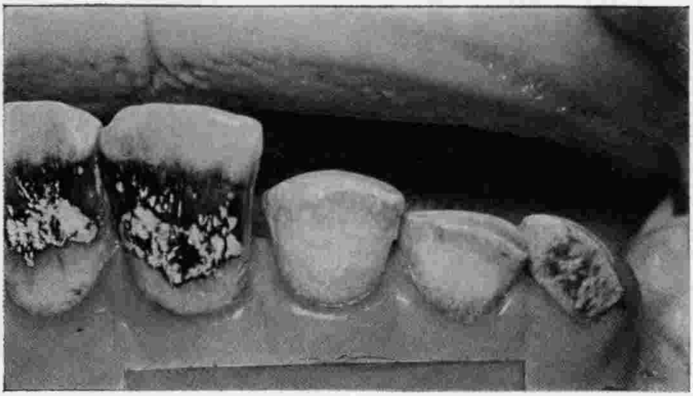
GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—light brown, *Wear*—(uppers) slight. SECOND and THIRD PAIRS: *Staining*—light brown, *Wear*—medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) heavy.

LOT NO. 11

Control Ration + 100 ppm Fluorine added as NaF and 0.5 Percent Defluorinated Phosphate (Total F = 107 ppm + Def. P)

Cow No. 25



Cow No. 12

Cow No. 70

ANIMAL NO. 20

CENTRALS: *Chipped Cap; Luster*—fair at cap; *Chalkiness*—cross, upper ⅛ porcelain, rest excessive; *Staining*—light brown, heavy; *Caries* and *Erosions*—(left) focal, medially, slight; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—(left) suspected to slight, (right) suspected; *Wear*—medium; *Classification*—(left) 4 to 5A, (right) 4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—slight; *Wear*—slight; *Classification*—4 to 5A.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—patch, slight; *Wear*—slight; *Classification*—5A.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: First Pairs: *Staining*—light brown, *Wear*—(uppers) slight. Second and Third Pairs: *Staining*—brown, *Wear*—medium. Fourth Pairs: Normal. Fifth Pairs: *Wear*—(uppers) medium and long centrally. Sixth Pairs: *Staining*—brown, *Wear*—(uppers) excessive and (lowers) medium.

The fluorine ingested by Lot 10 came from hay produced in the vicinity of an aluminum smelting plant. On a milligram per kilogram of body weight basis, Lot 10 was fairly comparable to Lot 5 for the first one and one-half years. For the total period reported, Lot 10 was a little above Lot 4 on a milligram per kilogram of body weight basis. Comparing the effects on teeth, Lot 10 was between Lots 3 and 4.

A comparison of Lot 10 with Lots 1, 2, 3, and 4 on the basis of feed consumption, weights and gains, and reproduction and calf records showed no appreciable differences. In the period of October, 1949, to October, 1950, there was a difference between Lot 10 and Lot 1 which was primarily attributable to one animal in Lot 10. This difference was not significant for any other period or for the total period reported. On the basis of fluorine content of urine, Lot 10 was between Lots 3 and 4.

On the basis of the data presented under the conditions of this experiment on the same milligram per kilogram of body weight basis, the fluorine in forage from an area near an aluminum smelting plant did not produce as severe tooth effects as the same level fed as NaF.

LOT NO. 11 (107 ppm F + Def. P)

ANIMAL NO. 25

CENTRALS: *Luster*—fair at cap; *Chalkiness*—cross, focal, excessive; *Staining*—light brown to black, heavy; *Caries*—multiple pinpoint and pinhead; *Erosions*—superficial in center and lower ⅓; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive, vegetative; *Enamel hypoplasia*—diffuse, patch, heavy; *Tooth hypoplasia*—medium; *Wear*—slight to medium; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, excessive, vegetative; *Enamel hypoplasia*—shell, heavy to excessive; *Tooth hypoplasia*—medium; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—shell, heavy to excessive; *Tooth hypoplasia*—medium; *Wear*—medium; *Classification*—5C.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) slight. SECOND PAIRS: *Staining*—brown, *Wear*—(uppers) medium, (lowers) slight. THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy, (lowers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—(uppers) medium, long posteriorly. SIXTH PAIRS: *Staining*—(lowers) brown-black, *Wear*—(uppers) excessive and (lowers) excessive and long posteriorly.

ANIMAL NO. 12

CENTRALS: *Luster*—upper ⅓ good; *Chalkiness*—cross, porcelain at cap, heavy; *Staining*—light brown to black centrally, heavy; *Erosions*—superficial, deep and undermining centrally; *Classification*—3.

INTERMEDIATES AND LATERALS: *Chalkiness*—excessive; *Staining*—yellow to light brown, excessive with dark brown in corners at cap; *Enamel hypoplasia*—heavy to excessive; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light to dark brown, excessive; *Enamel hypoplasia*—shell, patch, heavy to excessive; *Tooth hypoplasia*—slight to medium; *Wear*—medium; *Classification*—5C.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) slight and (lowers) medium. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy and (lowers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—(uppers) medium. SIXTH PAIRS: *Staining*—brown and black, *Wear*—(uppers) excessive and (lowers) medium and long posteriorly.

ANIMAL NO. 70

CENTRALS: *Luster*—fair at cap; *Chalkiness*—excessive; *Staining*—light to dark brown, excessive; *Erosions*—superficial, undermining, medium lower part of upper ½; *Enamel hypoplasia*—patch, lower ⅓ slight; *Classification*—5A.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—yellow to brown, excessive; *Enamel hypoplasia*—shell, heavy; *Tooth hypoplasia*—heavy; Wear—medium; *Classification*—5C.

LATERALS: *Chalkiness*—excessive; *Staining*—yellow to light brown, excessive; *Enamel hypoplasia*—shell, heavy; *Tooth hypoplasia*—heavy; *Wear*—medium; *Classification*—5C.

CORNERS: *Chalkiness*—excessive; *Staining*—light and dark brown; *Enamel hypoplasia*—shell and patch, heavy; *Tooth hypoplasia*—slight to medium; *Wear*—slight; *Classification*—5C.

GINGIVAE: Slight gingivitis.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) medium. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) heavy and (lowers) medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Staining*—brown, *Wear*—(uppers) medium and long posteriorly. SIXTH PAIRS: *Staining*—brown-black, *Wear*—(uppers) excessive, long posteriorly and (lowers) heavy and long posteriorly.

Small animal data showed that an excess of defluorinated phosphate (excess of calcium and phosphorus) would partially alleviate the effects of excessive fluorine ingestion.

A comparison of Lot 11 (107 ppm F plus 0.5 percent defluorinated phosphate) with Lot 8 showed that the effects on the intermediates, laterals and corners and premolars and molars were similar to or slightly more pronounced than effects on teeth in Lot 8. However, Lot 11 received more milligrams of fluorine per kilogram of body weight.

The average daily feed consumption of Lot 11 was significantly lower than that of Lot 1 but higher than Lot 8; however, the latter difference was not statistically significant.

Lot 11, considering the number of calves born, made a greater gain than Lot 8. Also, Lot 11 had a better reproduction record than either Lot 8 or 7.

The fluorine content of the ribs, as determined by rib biopsies, indicated that the amount of fluorine stored in the rib was greater for Lot 11 than for Lot 8.

Considering all these factors, the addition of 0.5 percent defluorinated phosphate to the rations of Lot 11 appeared to be beneficial. This is indicated by the increased production over Lot 8 which received a similar level of fluorine without the defluorinated phosphate.

LOT NO. 16 (Control Pasture, 10.6 ppm F; and Hay, 8.0 ppm F)

ANIMAL NO. 37

CENTRALS: *Luster*—very good; *Staining*—vegetative, slight; Longitudinal grooves; *Classification*—1A.

INTERMEDIATES: *Luster*—very good; *Staining*—vegetative, slight; *Classification*—1A.

LATERALS AND CORNERS: *Luster*—good; *Staining*—vegetative, slight; Enamel slightly rough lower ⅓; *Classification*—1A.

PREMOLARS AND MOLARS: All normal except (upper) third slightly short.

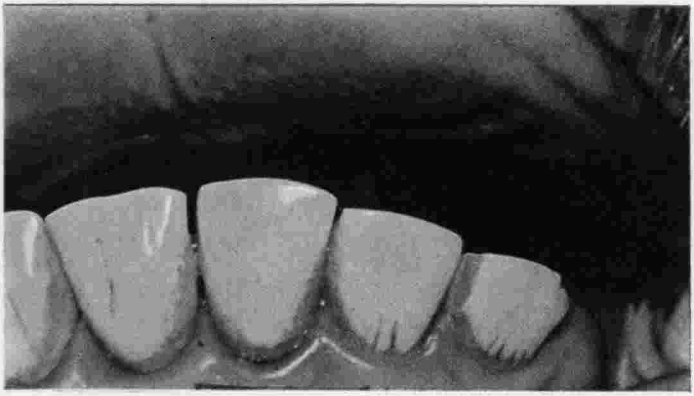
ANIMAL NO. 53

CENTRALS: *Luster*—very good; *Staining*—vegetative, slight; teeth separated 2 to 3 mm.; *Classification*—1A.

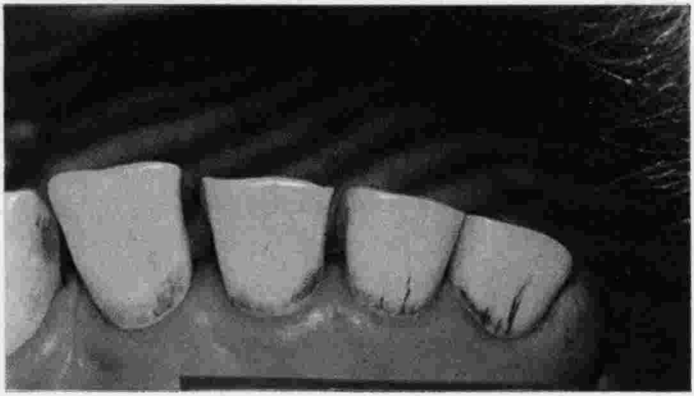
LOT NO. 16

Control Pasture

Pasture	10.6 ppm
Hay	8.0 ppm



Cow No. 37



Cow No. 53

Cow No. 65

INTERMEDIATES: *Luster*—good; *Chalkiness*—milky plaque right upper ½ centrally; *Staining*—vegetative, slight; *Wear*—slight; *Classification*—1A.
LATERALS AND CORNERS: *Luster*—good; *Chalkiness*—focal, slight; *Staining*—vegetative, slight; Enamel lower ⅓ rough; *Classification*—1A.
PREMOLARS AND MOLARS: Normal wear and stain.

ANIMAL NO. 65

CENTRALS: *Luster*—very good; *Classification*—1A.
INTERMEDIATES: *Chipped Cap*—(right) medially; *Luster*—very good; *Chalkiness*—focal, slight; *Staining*—vegetative, slight; *Classification*—1A.
LATERALS: *Chipped Cap*—(left) medially; *Luster*—good; *Chalkiness*—focal, slight; *Staining*—vegetative, slight; enamel lower ⅓ slightly rough; *Classification*—1A.
CORNERS: *Luster*—good; *Chalkiness*—(right) focal, slight, milky plaque lower ⅓; *Staining*—vegetative, slight; *Classification*—1A.
PREMOLARS AND MOLARS: Normal wear and stain.

Lot 16, the control pasture group, grazed pasture that had an average of 10.6 ppm fluorine, and received hay that averaged 8.0 ppm fluorine, from May, 1948, to October, 1952.

Chipped caps, longitudinal grooves, vegetative staining, and milky plaques were found in the apparently normal teeth of the cows in Lot 16.

A comparison of the teeth of Lot 16 with the teeth of Lot 1, control barn-fed lot, showed that the teeth of cows in Lot 16 had more luster. A comparison of the weights and gains, reproduction and calf records between the cows in Lots 14 and 16 showed no appreciable differences, although there is an obvious difference in teeth between the lots.

The fluorine content of the bones of calves weaned from this group was higher than the fluorine content of bones from calves weaned from barn-fed groups. The calves grazed on the control pasture during the summer season which probably accounts for this difference.

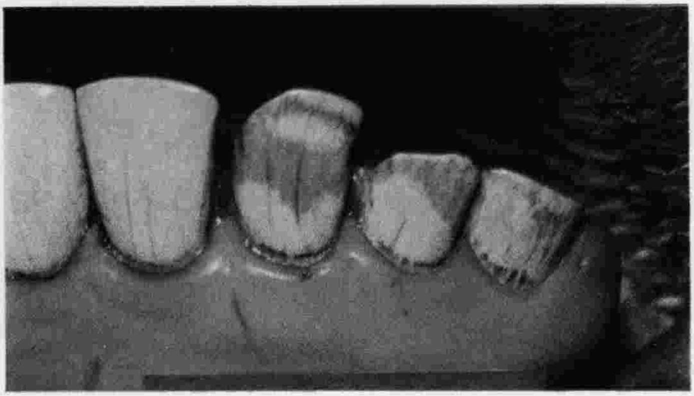
LOT NO. 15 (B_1 Pasture, 24.9 ppm F; and Hay, 40.0 ppm F)

ANIMAL NO. 3

CENTRALS: *Luster*—good; *Chalkiness*—focal; *Staining*—suspicious discoloration lower ½, vegetative; Longitudinal Grooves; *Classification*—1B.
INTERMEDIATES: *Luster*—good at cap and lower ⅓; *Chalkiness*—focal; Longitudinal Cracks; *Staining*—yellow to light brown centrally, heavy, vegetative; *Wear*—uneven, slight; *Classification*—2.
LATERALS: *Luster*—fair; *Chalkiness*—diffuse; *Staining*—yellow to light brown, medium, vegetative; *Enamel hypoplasia*—pit, slight; *Wear*—medium; *Classification*—4.
CORNERS: *Chalkiness*—focal, diffuse, porcelain, slight; *Staining*—yellow to light brown, heavy, vegetative; *Enamel hypoplasia*—suspicious; Enamel rough; *Classification*—4.

LOT NO. 15

Pasture 24.9 ppm F
Hay 40.0 ppm F



Cow No. 3

Cow No. 59

Cow No. 41

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—light brown. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) slight. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Wear*—(uppers) excessive to gum line and (lowers) slightly long anteriorly and centrally and short posteriorly.

ANIMAL NO. 59

CENTRALS: *Luster*—upper ½ good, lower ½ fair; *Chalkiness*—focal, cross, porcelain, heavy; *Staining*—medium, vegetative, light brown; *Caries*—(right) pinhead medial central; *Classification*—(right) 3, (left) 2.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown, excessive; *Enamel hypoplasia*—slight; *Wear*—uneven, medium to heavy; *Classification*—4.

LATERALS: *Chipped Cap; Chalkiness*—cross, porcelain, heavy; *Staining*—yellow to light brown, medium, vegetative; *Erosion*—(left) upper ½ medial, slight, black staining; *Wear*—slight; Longitudinal Crack (left) dark staining; *Classification*—(right) 2, (left) 3.

CORNERS: *Chalkiness*—diffuse; *Staining*—slight, vegetative, light brown; *Enamel hypoplasia*—pit, slight; *Wear*—slight; *Classification*—4.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—slight to medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Wear*—(uppers) excessive, nearly to gum line and (lowers) long posteriorly, sheer anteriorly.

ANIMAL NO. 41

CENTRALS: *Luster*—good upper ½; *Chalkiness*—cross; *Staining*—light brown lower ½, slight, vegetative; Longitudinal Cracks stained; *Classification*—2.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—light brown to black, heavy; *Erosions*—(right) superficial centrally; *Enamel hypoplasia*—patch, slight; *Wear*—heavy uneven; Longitudinal Cracks; *Classification*—5A.

LATERALS: *Chalkiness*—diffuse, excessive; *Staining*—light brown, medium; *Enamel hypoplasia*—pit, slight; *Wear*—slight to medium; Longitudinal Cracks; *Classification*—4.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; *Enamel hypoplasia*—slight to medium; *Classification*—5A.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown. SECOND and THIRD PAIRS: *Staining*—(uppers) brown and (lowers) dark brown, excessive, *Wear*—(uppers) medium and (lowers) slight. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—brown, excessive, *Wear*—(uppers) excessive centrally and (lowers) excessive anteriorly and long posteriorly.

The pastures grazed by Lot 15 had an average of 24.9 ppm fluorine for the period from May, 1948, to October, 1952. Lot 15 received hay that averaged 40 ppm fluorine for the entire period; however, the hay fed during the winter of 1948–49 averaged 59.8 ppm fluorine. The intermediate teeth of Cows 59 and 41 and laterals of Cow 3 apparently show effects caused by hay that averaged 59 ppm fluorine, compared to a range in fluorine content from 60 to 15 ppm for the remaining period. While the teeth show the effects of fluorine, they apparently are not affected as much as Lot 4 (37 ppm F), in the barn-fed group but more than Lot 3, which received 27 ppm fluorine. In general, calves from cows in Lot 15 had a higher bone fluorine content than calves from the barn-fed groups. This was a result of the calves grazing forage which contained fluorine, and is further evidence that there was relatively little transfer of fluorine to the fetus or through the milk at ingested fluorine levels studied in Experiment I.

A comparison of the cows in Lot 15 with the control pasture, Lot 16, showed that there was no appreciable difference in the final weight and gains for the period reported. Lot 16 made greater gains during the first two periods, and Lot 15 made greater gains during the last three periods. This can be explained partially by the fact that pasture conditions were less favorable for Lot 15 during the summers of 1948 and 1949. A comparison of the reproduction and calf records shows no appreciable difference for the period reported, although the record of Lot 16 was slightly better due to conditions of the 1949 season. On the contrary, Lot 15 had the better record for the 1951 and 1952 seasons.

LOT NO. 14 (B_2 Pasture, 44.9 ppm F; and Hay, 51.0 ppm F)

ANIMAL NO. 43

CENTRALS: *Luster*—fair to good; *Chalkiness*—focal, porcelain, cross in upper ½ and diffuse in lower ⅓; *Staining*—vegetative, very light yellow in lower ⅔; *Caries*—pinhead foci lower ⅓; *Classification*—3.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—focal, light to dark brown, heavy; *Caries* and *Erosions*—central ⅓ medially; *Enamel hypoplasia*—pit and patch, slight; *Wear*—uneven and short medially, heavy; *Classification*—4.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown, focal, heavy; *Enamel hypoplasia*—pit, patch, slight; *Wear*—medium to heavy; *Classification*—4.

CORNERS: *Chalkiness*—excessive; *Staining*—lower ½ light brown and upper ½ dark brown, excessive; *Enamel hypoplasia*—pit, slight; *Wear*—medium; *Classification*—5A or 4.

GINGIVAE: Normal.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—dark brown. SECOND and THIRD PAIRS: *Staining*—dark brown, *Wear*—(uppers) heavy, (lowers) medium. FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—dark brown, *Wear*—(uppers) heavy and (lowers) sheer anteriorly and long posteriorly.

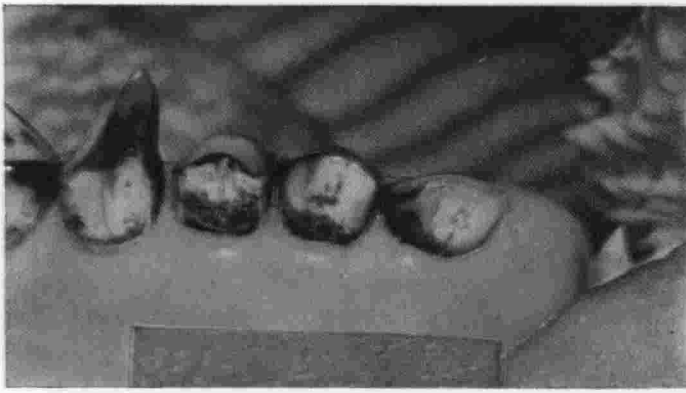
LOT NO. 14

Pasture 44.9 ppm F
Hay 51.0 ppm F

Cow No. 43

Cow No. 56

Cow No. 71



ANIMAL NO. 56

CENTRALS: *Luster*—good at cap; *Chalkiness*—cross, porcelain, focal upper ¼, lower ⅔ excessive; *Staining*—light to dark brown, focal, medium, vegetative; *Enamel hypoplasia*—suspicious; *Wear*—(left) medium, sheer medially; *Classification*—4.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—excessive, vegetative, focal, light brown; *Enamel hypoplasia*—pit, slight; *Wear*—heavy; *Classification*—5A.

LATERALS: *Chalkiness*—excessive; *Staining*—vegetative, light brown, focal, medium; *Enamel hypoplasia*—focal, pit, slight; *Wear*—medium; *Classification*—4.

CORNERS: *Chalkiness*—excessive; *Staining*—light brown to brown, excessive; *Enamel hypoplasia*—patch, suspicious to slight; *Wear*—slight; *Classification*—4.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) slight. SECOND and THIRD PAIRS: *Staining*—brown, *Wear*—medium. FOURTH PAIRS: Normal. FIFTH PAIRS: *Wear*—(uppers) heavy centrally and long posteriorly, (lowers) normal. SIXTH PAIRS: *Staining*—brown, *Wear*—(uppers) medium and (lowers) medium anteriorly and long centrally.

ANIMAL NO. 71

CENTRALS: *Chalkiness*—excessive; *Staining*—excessive, vegetative, upper ½ brown-black and lower ½ light brown; *Erosions*—superficial and undermining upper ⅔; *Enamel hypoplasia*—suspicious; *Wear*—uneven, short medially, heavy; *Classification*—4.

INTERMEDIATES: *Chalkiness*—excessive; *Staining*—excessive, vegetative, light brown to black; *Enamel hypoplasia*—lower ½ pit, slight to medium; *Wear*—uneven, heavy; *Tooth hypoplasia*—suspicious; *Classification*—5A.

LATERALS: *Chalkiness*—excessive; *Staining*—light brown to black, excessive; lower part vegetative; *Enamel hypoplasia*—focal, pit, slight; *Wear*—uneven, medium to heavy; Longitudinal Cracks—dark brown stained; *Classification*—5A or 4.

CORNERS: *Chalkiness*—excessive; *Staining*—yellow to brown with (right) cap black, excessive; *Caries* and *Erosions*—medially gingival ⅓, slight; *Enamel hypoplasia*—focal, pit, slight; *Wear*—slight to medium; *Classification*—5A or 4.

PREMOLARS AND MOLARS: FIRST PAIRS: *Staining*—light brown, *Wear*—(uppers) medium. SECOND PAIRS: *Staining*—brown, *Wear*—medium to heavy. THIRD PAIRS: *Staining*—brown, *Wear*—medium to heavy; FOURTH and FIFTH PAIRS: Normal. SIXTH PAIRS: *Staining*—(lowers) brown, *Wear*—(uppers) excessive and (lowers) medium to heavy anteriorly.

Lot 14 cows grazed pastures and were fed hay that had a weighted average of 47 ppm fluorine. A comparison of Lot 14 with Lot 16 showed that there was no appreciable difference in the final weight, average daily

TABLE 14.—CLASSIFICATION SUMMARY OF INCISOR TEETH OF COWS IN EXPERIMENT I, LOTS 1–11 AND 14–16 [a]

Lot no.	Total F in ration ppm	Animal no.	Centrals 1st pair C	Inter-mediates 2nd pair C	Laterals 3rd pair C	Corners 4th pair C
1	7	42	1A	1A	1A	1A
		13	1A	1A	1A	1A
		24	1A	1A	1AX	1A
2	17	46	1A	1A	2	1B
		16	1A	2	2	4
		11	2	2	L-2 R-4	5A
3	27	32	L-2 R-3	3	4	4
		47	1A	2	3	4
4	37	48	2	4	5A	5A
		30	3	4	5A	5A
		31	3	4	5B	5B
5	47	49	L-2 R-1A	4	5A	5B
		23	3	4	5B	5B
		9	3	5A	5B	5B
6	57	50	3	4	5A	5B
		6	3	4	5A	5B
		1	4	5B	5B	5B
7	77	2	3	5C	5C	5C
		58	5A	5C	5C	5C
		21	5A	5C	5C	5C
8	107	28	5A	5C	5C	5C
		29	5A	5C	5C	5C
		61	5A	5C	5C	5C
9	B_1 Hay	27	3	5A	4	4
		26	2	2	4	4
		63	2	4	4	4
10	B_2 Hay	19	2	4	2	2
		67	1A	4	5A	5A
		20	3	L-5A to 4 R-4	5A to 4	5A
11	107 + Def.	25	3	5C	5C	5C
		12	3	5C	5C	5C
		70	5A	5C	5C	5C
14	B_2 Pasture	43	3	4	4	5A or 4
		56	4	5A	4	4
		71	4	5A	5A or 4	5A or 4
		40	5A	5A	5A	5A
		57	3	4	4	5A
15	B_1 Pasture	3	1B	2	4	4
		59	L-2 R-3	4	L-3 R-2	4
		41	2	5A	4	5A
		52	2	4	4	4
		33	3	4	4	4
		55	3	4	4	4
16	Control	37	1A	1A	1A	1A
		53	1A	1A	1A	1A
		65	1A	1A	1A	1A
		44	1A	1A	1A	1B
		14	1A	1A	1B	1A
		38	1A	1A	1A	1A

[a] Numerical rating of incisor teeth is based on preceding plates, nomenclature and classification of Table 15.

gains, and gains of calves produced for the period reported. The teeth of cows in Lot 14, compared to teeth of cows in Lot 16 obviously were more affected by fluorine.

The irregular production of one cow in Lot 14 accounts for the slight difference in percent of calves produced between Lots 14 and 16.

A comparison of Lot 14 with the barn-fed group, Lot 5 (47 ppm F), which is most comparable on the basis of ppm of fluorine in the feed, revealed that the teeth of Lot 5 were slightly more affected by fluorine. A study of the teeth readings indicated that the teeth of cows on pasture, Lot 14, were more comparable to those of Lot 4 of the barn-fed lots. Lot 14 received fluorine averaging approximately 53 ppm for the period of May, 1948, to April, 1950, while Lot 4 averaged about 37 ppm. It appears that fluorine from the pasture forage in the vicinity of the aluminum smelting plant apparently affected the teeth less than did a similar level of fluorine fed as NaF in dry lot.

A comparison of pasture groups with the barn-fed groups, shows that the pasture groups had more tooth wear than the barn-fed groups under conditions of this test.

NOMENCLATURE

Fluorosis as defined in medical literature specifically indicates the development of chronic toxicosis or poisoning as the result of ingestion of fluorine in amounts above normal. Recently the term fluorosis in animals has been used somewhat loosely to denote mottling and minor structural defects in the incisor teeth, as well as severe effects in animals. The term fluorosis used in connection with effects on teeth should be described as dental fluorosis.

Literature surveys and discussions with co-workers have made it apparent that there is a need for some standardization of terms to describe the various effects of dental fluorosis in cattle. Observations on cattle, Experiment I and others, fed various levels of fluorine over a period of years has provided the basis for the descriptive terms used in this discussion.

This report on nomenclature is a result of collaborative effort by workers at the University of Tennessee, Paul H. Phillips of the University of Wisconsin, and L. P. Doyle of Purdue University, and experiences and interpretations of numerous visiting workers in the field. The Tennessee study includes cattle that have been barn-fed definite levels of fluorine for five years or more, and findings from field surveys of numerous animals ingesting increased amounts of fluorine. An attempt has been made to define and standardize terms that can be used in describing effects of fluorosis.

It is anticipated that, as the present research with beef and dairy cattle and sheep progresses in Tennessee, Wisconsin, Utah, California and other stations, these terms may be further standardized. Other terms may be added, as these studies progress, that will assist in general data interpretations.

Normal Cattle Teeth

A study of cattle teeth to determine symptoms and effects of increased fluorine ingestion must take into account certain characteristics peculiar to the teeth of all cattle. It is important that normal occurrences not be mistaken for abnormal developments. Such factors as the number of permanent teeth, chips and cracks in normal enamel, vegetative staining, and normal looseness of incisors appear to be most deceptive to persons unfamiliar with normal characteristics of cattle teeth. Since dental fluorosis in cattle seems to appear only in permanent teeth, only brief consideration will be given here to temporary teeth.

At birth, or shortly thereafter, the calf usually is equipped with 8 small "front" teeth or incisors and 12 cheek teeth. These cheek teeth—premolars—are arranged with three on each of the upper and lower jaws of each side. Temporary incisors, located only on the mandible, are much smaller than the permanent teeth. The temporary premolars, with the exception of the lower first cheek tooth on each side have a larger grinding area than the permanent set of premolars.

There are no "baby" or temporary molars. Ordinarily, the first molar, the fourth cheek tooth from the front of the mouth, erupts when the animal is 4 to 6 months of age. For about 6 to 12 months, the temporary premolars and first molar constitute the group of cheek teeth. The second molar, according to Sisson (1930), erupts when the animal is about 12 to 18 months old, and the third molar at 24 to 30 months.

Sisson states that the first permanent premolar erupts when the animal is 18 to 24 months of age and the second at 24 to 30 months. In field surveys conducted by this station, it has been observed that the second premolar may erupt before the first. The third premolar may erupt earlier than 30 to 36 months of age as stated by Sisson.

Certain differences are observed between permanent premolars and molars of the upper jaw and those of the lower jaw. The tooth below often appears before its counterpart in the upper jaw. Observations at this station indicate that this applies particularly to the second and third molars and second and third premolars.

It is also commonly observed that the cheek teeth of the upper jaw appear shorter than those below. This is characteristic of the molars. In examining teeth for wear, the examiner should not confuse the normal shortness of the upper molars with abnormal wear predisposed by any metabolic disturbance.

Often there appears to be an exception to this observation that mandibular (lower) molars appear longer than maxillary teeth. In some individual animals the anterior two-thirds of the lower fourth molar is much shorter than either the third premolar or the adjoining molar. This change has been observed in cattle with no evidence of dental fluorosis.

Both the upper and lower cheek teeth are heavily black stained in the gingival one-third or one-half. The lower first premolar usually is completely stained. Such black staining appears in the mouths of cattle observed at this station. It is found in mouths showing no evidence of clinical dental fluorosis. The examiner must differentiate this from abnormal types of staining in evaluating symptoms of dental fluorosis.

Observations in Tennessee agree generally with Sisson's "Table of Average Periods of Eruption of the Teeth in the Ox," if the period of initial rupture of the mucosa is considered as the date of eruption:

Permanent

First incisor (centrals)	1½ to 2 years
Second incisor (intermediates)	2 to 2½ years
Third incisor (laterals)	3 years
Fourth incisor (corners)	3½ to 4 years

The permanent incisors follow a definite pattern of eruption but an indefinite schedule for this process. It is also noted that one member of a pair may reach full height before the corresponding tooth has erupted.

Preliminary studies indicate that there is a difference in eruption dates between breeds. There may be a difference between areas and between farms, depending upon the nutrients in the ration and level of feeding.

Normal incisors of cattle are imbedded in a way that permits considerable movement.

Certain blemishes and slight injuries occur in incisors so frequently that they must be considered part of the normal bovine dental pattern. Such defects include chipped caps, longitudinal cracks, grooves, vegetative staining, "milky plaques," rough enamel, and exposed pulp cavities.

The hardness of enamel on sound incisors of cattle and the feeding habits of the animals appear to affect chipping at the cap of the teeth. Some chips normally are seen on the knife-like anterior edge of incisors with no wear, or very slight wear. These chips are seldom more than two millimeters deep. Consequently, they become smooth and disappear quite rapidly with the normal amount of tooth wear.

Minute longitudinal cracks appear frequently in teeth unaffected by an excess of fluorine. In many cases, the fissures or cracks extend from the cap almost to the neck of the tooth. The fissures may be shallow or may penetrate to the dentine. Studies at the Tennessee Station indicate that these longitudinal cracks should be considered characteristic of apparently normal teeth.

Juices of grass, hay, and other feeds often cling to the enamel of incisors. The resulting vegetative staining is seen most frequently on newly erupted teeth. The forage juices adhere to, or perhaps penetrate, the enamel cuticle. Grass juices also may cling to the corner teeth, the enamel of which often remains rough for many months after eruption of the teeth.

The enamel of a recently-erupted incisor often appears rough. In the case of centers, intermediates and laterals, this roughness often disappears with the wearing away of the cuticle. In many corner teeth, however, the enamel retains a rough appearance throughout life. This roughness must be distinguished from certain types of hypoplasia.

Animals at the Tennessee Station, on control rations (containing an average 2 to 15 ppm fluorine), frequently show a lesion, described as a milky plaque, on lateral and corner incisors. Occasionally the plaque occurs on the intermediates. This lesion is possibly a manifestation of enamel hypoplasia. Observations made in this study indicate that the appearance of a milky plaque on an incisor in no appreciable way shortens the functional life of that tooth.

Pulp cavities of incisors are often found exposed in teeth not affected by fluorine. The exposed cavities, which seem quite common in cattle, six or eight years old, appear to cause no distress. It is only rarely that a tooth with the exposed pulp cavity appears to die. After the pulp is exposed, a secondary dentinal occlusion of the cavity occurs.

Dental Fluorosis in Cattle

The enamel-forming organ is remarkably sensitive to disturbances during the periods of matrix formation and calcification. The effects of excessive amounts of fluorine upon calcification are recognized. Relatively small amounts of dietary fluorine are capable of producing permanent changes in the enamel. Changes due to fluorine probably do not occur in temporary teeth. This is due chiefly to the advanced state of development of deciduous teeth at birth and to the ability of the placental structures to restrict the wholesale transfer of fluorine from the mother to the fetus. The effects caused in teeth by toxic quantities of fluorine occur during the formative stages (dentinogenesis, amelogenesis, and calcification). Since the developmental stages of tooth formation are cyclic and rhythmic with periods of activity and rest, and as the concentrations of the toxic substances to which these extremely sensitive tissues are exposed are necessarily subject to variation, irregular areas of involvement, or somewhat bizarre patterns of affection, may be formed. Changes in pairs of teeth are usually bilateral but, not uncommonly, only one tooth of a pair may be affected. The development and subsequent eruption of one of a pair of teeth may precede its counterpart by three, four, or more months.

In these studies, some anomalies, such as the various types of chalkiness, intra-enamel staining, and the various types of enamel hypoplasia have been observed at the time the erupting tooth became visible. Subsequent studies of the same tooth verified the initial observations.

The toxic effect of fluorides, as exerted upon the teeth and as reflected by severe and progressive dental pathology, may or may not be correlated with the influence of the fluoride upon the overall clinical and economic injury to the animal.

In general, the following descriptive classifications have been proposed in the order of their ascending gross structural importance. The degree of change for each class is evaluated as slight, medium, heavy, and excessive.

I. *MINOR ENAMEL IMPERFECTIONS*

A. *Chalkiness or Mottling:* Areas of dull or flat white color interspersed in and replacing the smooth, lustrous enamel. The change is probably the first indication of a small increase in the quantity of fluorine in the ration or water. A small amount of fluorine continuously ingested may be reflected by an increase in the extent of chalkiness of successively erupting teeth. These areas may be observed in the teeth of apparently normal animals.

1. *Focal chalky*—a few discrete focal chalky areas. Numerous discrete foci may present a stippled effect.

2. *Cross chalky*—small, transverse chalky bars having a fairly regular pattern of distribution. The bars are sharply defined from the surrounding normal-appearing enamel.

3. *Chalky striations*—distinct chalky bands of transverse or vertical alignment, which may be designated as (a) cross, or (b) longitudinal, respectively.

4. *Porcelain mottling*—narrow refringent steel blue or grayish lines transecting normal-appearing enamel. Less frequently observed are concentrically arranged porcelain configurations.

5. *Diffuse chalky*—the chalky foci are increased in number and distribution. These may present a uniformly slight to medium dull appearance.

6. *Excessive chalkiness or opaqueness*—the enamel is uniformly and completely dull and lusterless.

B. *Milky Plaques:* Dull white, grayish white, or creamy rhomboidal or triangular shaped intra-enamel areas. Their location is usually near the gingival margin. These anomalies frequently occur in the teeth of normal cattle. Plaques occur most often in lateral teeth, to a lesser extent in corners and intermediates, and seldom in centers. In many instances rectangular or ovoid milky, creamy, or light brown stained areas with a darker stained central portion have been observed in the enamel of apparently normal teeth of adult cattle that were not affected by excessive fluorine ingestion.

Caries or erosions have not developed in these teeth as they have occurred in stained areas in fluorotic teeth.

II. *STAINING*

A. *Intra-enamel Staining:* The normal color of the enamel in a tooth is variegated by yellow, brown, or black stains. These areas are less resistant to erosive forces when increased amounts of fluorine are involved.

1. *Focal staining*—small areas of intra-enamel stain.

2. *Diffuse staining*—several areas of focal intra-enamel staining which may involve most of the enamel of a tooth.

3. *Cross staining*—stained transverse bands.

4. *Longitudinal staining*—stained vertical bands.

B. *Vegetative Stain:* An extraneous stain derived from feeds or other materials. Most of this pigment can be removed by the careful use of a detergent.

The gingival half to two-thirds of the premolar and molar teeth are normally covered with a firmly adherent dark brown to black calculus. This superficial accumulation frequently presents a metallic luster. In the case of fluorotic teeth the parts free of calculus, including the attritional surfaces, may be brown to black stained.

C. The amount of staining may be described as follows:

1. Slight staining—about ⅛ of the tooth surface is stained.

2. Medium staining—about ¼ of the tooth surface is stained.

3. Heavy staining—approximately ½ of the tooth surface is stained.

4. Excessive staining—approximately ¾ or more of the tooth surface is stained.

III. *DENTAL CARIES*[a]—Dissolution and disintegration of the enamel and/or dentine.

A. *Caries:*

1. *Pre-carious foci*—variable in number and small, may partially or completely penetrate the enamel; stained slightly brownish or black.

2. *Caries, Pinpoint to Pinhead*—foci that are usually progressive.

B. *Erosion:* The loss of enamel substance from the surface of the tooth, exclusive of attrition or apposition. The enamel may be superficially or deeply affected. The partially-eroded enamel and/or exposed dentine are usually stained dark brown to black. These lesions develop from progressive erosion of pre-carious foci and caries.

Occasionally unilateral lesions which appear to be carious are observed

[a] Caries, as seen in this work, differs from the definition of that term in human dentistry inasmuch as the enamel, and apparently not the dentine and pulp, is involved in animals.

in the incisors of an animal that presumably has not ingested fluorine in excess of that normally present in feeds. These lesions apparently are non-specific developmental anomalies which are surrounded by lustrous enamel.

The enamel adjacent to carious and erosive foci and vertical enamel fissures in fluorotic teeth is frequently grayish in color and represents the initial changes of the enamel caries. As this lesion progresses there is an extension of the carious and erosive processes, and an increased staining to a dark brown or black.

1. *Types of erosion:*

a. Erosion—may be deep or superficial, involving the surface of the enamel.

b. Undermining erosion—originates at the base of a deeply penetrating carious lesion and spreads peripherally. An apparent superficial shell of enamel, which may become stained grayish black, covers the progressive underlying erosive lesion until detached by mechanical forces or by mass erosion. This occurs with continued ingestion of fluorine. The second and third premolar and third molar teeth may be affected with multiple pinhead carious lesions.

2. *The status of enamel erosion* may be designated as follows:

a. *Slight erosion*—approximately ⅛ of the area of the anterior enamel of the tooth is eroded.

b. *Medium erosion*—about ¼ of the area of the anterior enamel is eroded.

c. *Heavy erosion*—about ½ of the area of the anterior enamel is eroded.

d. *Excessive erosion*—¾ or more of the area of the anterior enamel is eroded.

IV. *HYPOPLASIA*—defective or incomplete formation of the dental structures. Hypoplastic defects may occur in the enamel, or in both the enamel and the entire tooth.

A. *Enamel hypoplasia*—has many different manifestations. It may be present in part or all of the enamel.

1. *Pit hypoplasia*—the failure of enamel substance to develop fully results in the formation of small fovea or pits on the surface of the tooth. The pits are surrounded by more fully developed enamel. Typically, these pits are arranged in transverse rows, but may be diffuse. They are usually pinpoint to pinhead in size.

2. *Patch hypoplasia*—irregularly alternating areas of ridges and depressions produced by enamel of normal thickness and abnormally thin enamel, respectively. The enamel is usually uniformly yellow-brown. Involvement may occur as a transverse band of varying width or the entire

labial surface may be affected. Carious foci frequently develop in the hypoplastic areas.

3. *Shell hypoplasia*—is manifested by the enamel being uniformly and abnormally thin. Enamel is uniformly yellow or light brown stained. Carious lesions are not frequently associated with this type of enamel hypoplasia.

The extent and degree of enamel hypoplasia may be described as slight, medium, heavy, or excessive.

4. *Other hypoplasia*—some other forms of enamel hypoplasia observed are (a) foamy enamel, (b) bleb-like formation, and (c) calcotraumatic bands.

B. *Tooth Hypoplasia*—this term applies to any faulty underdevelopment of the tooth. It may be manifested by (a) a simple reduction in size, (b) a decrease in the overall length with an apparent increase in the anterior-posterior thickness. This results in a stubby, malformed tooth, appreciably reduced in the lateral-medial dimension.

C. *Enamel and tooth hypoplasia*—may occur in the premolar and molar teeth. Enamel and tooth hypoplasia are most apparent in the second and third premolars and third molars.

V. *DENTAL WEAR*

Wear of normal teeth is fairly accurately correlated with the age of the animal, the type of nutrients ingested, and the environmental conditions. Variations in the size of teeth and their ability to withstand wear occur among individual animals. The influence of disease, nutritional deficiencies and the ingestion of toxic substances upon the development of teeth is recognized. The ingestion of toxic quantities of fluorine during the developmental stages of tooth formation results in an inferior tooth which may have poor wearing qualities. Tooth hypoplasia may complicate the establishment of an accurate estimation of amount of wear.

Teeth of normal composition may chip on contact with hard objects. Teeth which have been affected by excessive amounts of fluorine tend to crumble rather than chip.

In herds under presumably normal conditions, teeth will show a degree of wear from none to excessive, depending upon age when gauged by loss of the tooth crown height.

It was decided in these fluorine studies that only wear estimated to be in excess of normal, with consideration of age, condition, present and previous management of the animal, could be attributed to ingested fluorine. This is admittedly an arbitrary estimation. To be fairly accurate in his readings, the examiner must have a thorough knowledge of normal wear for the age of the animal, and the general environmental conditions and management of the herd. The degree of teeth wear resulting from the ingestion of fluorine above normal then may be arbitrarily classified with the following letters used to abbreviate the descriptive terminology.

A. Normal wear for age.

B. *Slight*—this represents a tooth with about ⅛ of the crown height worn off in excess of that considered normal for age.

C. *Medium*—this designates a tooth with about ¼ of the crown height worn off in excess of that considered normal for age.

D. *Heavy*—this includes a tooth with ½ the crown height worn off in excess of that considered normal for age.

E. *Excessive*—this designates a tooth with ¾ or more of the crown surface worn away in excess of that considered normal for age.

P. *Uneven wear*—this is used to describe a tooth with an uneven cutting edge.

R. *Tooth erupting*—this term is used to denote a tooth just piercing the gingiva. It is difficult to determine specific dental effects by observations before a tooth has reached maturity.

Special kinds of wear include:

1. *Rolling wear*—the labial and lingual aspects are excessively worn, resulting in the formation of a convex attritional surface.
2. *Bevel wear*—excessive wear at the dorsum of the labial surface.
3. *Appositional wear*—wear of incisor teeth as the result of friction and pressure with adjacent teeth. Caries and erosion of the enamel are likely to occur in the appositional wear areas in the teeth of cattle which have ingested increased amounts of fluorine.

Wear of premolar and molar teeth, due to their location, is somewhat more difficult to evaluate accurately. The first molar is less affected due to the ability of the placenta to limit the amount of mother-to-fetus fluorine transfer.

An increased intake of fluorine, environmental or experimental, results in a progression of dental effects. The changes from minor to severe effects in dental fluorosis are: (1) Chalkiness of the enamel. (2) Staining of the enamel. (3) Caries and erosion of the enamel. (4) Hypoplasia of the enamel. (5) Hypoplasia of the teeth. Wear of the incisor and cheek teeth is proportionately accelerated. It is usual for combinations of these changes to appear in the same teeth or in the same mouth. However, an excessive, constant increase of fluorine may result in a marked enamel, or enamel and tooth hypoplasia, with a masking of less severe effects.

The effects upon cattle teeth resulting from currently ingested fluorine above normal amounts, therefore, depend upon many factors, including:

1. The age of the animal and the stage of tooth development.
2. The level of fluorine ingested.
3. The length of time exposed to the increased fluorine ingestion.
4. The initial fluorine stored in the animal body.

Table 15.—Classification of the Effects of Dietary Fluorine on Teeth of Cattle

Classification	Wear greater than normal for age and conditions	Chalkiness or mottling (Incisors)					Staining	Caries and/or erosions	Hypoplasia		Relative effects greater than normal on each tooth
		Focal	Cross Chalky	Chalky striations-cross or longitudinal	Porcelain	Excessive			Enamel	Tooth	
1A	Depends on age and individual variations	**May be very slight Luster—good**									None
1B	Depends on age and individual variations	**Slight to medium Luster—good**			May be very slight		***May be suspiciously discolored***				None
2	Depends on age and individual variations	Slight to diffuse Luster—good to fair			Slight to medium		**Usually very slight to heavy brown**				Questionable to slight
3	Table surface may be good on cattle to 6 years of age	Slight to heavy Luster—good to fair			Slight to heavy		Usually slight to heavy	**Usually Pathognomonic**			Slight
4	Table surface may show negligible to medium wear	May show some of the above effects Lusterless			Slight to heavy	May be only partially	Slight to excessive brown and black stain	May be pre-carious or carious after 1 to 2 years in wear	**Suspected to slight**	None	Slight to medium
5A	Wear variable slight to medium	May show some of the above effects				May be partially	Slight to excessive brown and black stain	Progressive type of erosions may be present	**Slight to medium**	May be suspicious	Medium
5B	Wear variable slight to excessive					May be partially	Slight to excessive brown and black stain	Progressive type of erosions may be present	**Medium to heavy**	May be slight to medium	Medium to heavy
5C	Medium to excessive wear					May be partially	Slight to excessive brown and black stain	Progressive type of erosions may be present	**Heavy to excessive**	May be medium to excessive	Heavy to excessive

"X" when added to classification number denotes an abnormality other than those traceable to fluorine.
Boldface terms indicate symptoms which would definitely determine the classification of a tooth.

5. The solubility and availability of the fluorine material ingested.

Other factors, such as the level of nutrition, pregnancy, and lactation may accentuate or modify the effects of above normal amounts of fluorine ingested by cattle or sheep.

While the clinical symptoms of classical dental fluorosis are distinctive, it is emphasized that the general clinical condition of the animal does not necessarily correspond to or agree with the apparent effect of fluorine upon the teeth. The presence of dental fluorosis in cattle, literally interpreted, means only that fluorine was ingested in amounts sufficient to damage or to mark the teeth during the period that the affected teeth were developing. Despite this fact, tooth changes are of great value in diagnosis, especially when they are correlated with other symptomatic criteria of fluorosis.

Summary

Yearling Hereford heifers in this experiment were started on test in April and June, 1948. Lots 1–8 and 11 were fed a basal ration containing 7 ppm fluorine, with 10 to 100 ppm fluorine added as NaF. Lots 9 and 10 received no additional fluorine as NaF but were fed hays, which contained an increased F content, that were harvested in an area near an aluminum smelting plant. Lots 1–11 were individually fed in a barn. Lots 14 and 15 were grazed on two pastures containing forage with an increased fluorine content caused by their proximity to an aluminum smelting plant and Lot 16 was grazed on a control pasture.

Lots 7, 8, and 11 (receiving added F levels of 70 ppm, 100 ppm, and 100 ppm + defluorinated phosphate), after about 18 months on test, showed a significant decrease in feed consumption compared to Lot 1, controls. This decrease in feed consumption continued for the period reported. There was a statistically significant decrease in feed consumption for Lots 5 and 6 (40 ppm and 50 ppm added as NaF) compared to Lot 1 for the period of October, 1950, to October, 1951 (two and one-half to three and one-half years on test), which continued for the period reported. There was no appreciable difference between feed consumption of Lots 1, 2, 3, 4, 9, and 10 for the period from April, 1948, to October, 1952.

Considering the reproduction and calf records, there was no appreciable difference in weights and gains between any of these lots except that Lots 8 and 11 were lower than Lot 1. Although Lot 7 had a high final weight, it is likely that if this lot had raised a similar number of calves as Lots 1–6, 9, and 10, the weights would have been lower. A comparison of the weights and gains, reproduction and calf records between cows in Lots 14, 15, and 16 showed no appreciable difference. However, the differences in teeth effects are related to level of fluorine ingestion.

There was no apparent difference in reproduction or calf records except that Lots 7 and 8 were lower and Lot 11 was probably lower. A com-

parison of Lot 11 to Lot 8 showed that the addition of 0.5 percent defluorinated phosphate (Lot 11) to rations containing an added 100 ppm fluorine as NaF improved feed consumption and calf production.

The higher fluorine levels fed in Experiment I did not significantly affect the apparent digestibility under conditions listed in this experiment.

The fluorine stored in the body was directly related to the level of intake as shown by the fluorine balance studies. The fluorine content of the urine varied generally with the level of intake. Where differences between levels were small, individual variations within a given lot were often much greater than the variations between levels. When the level in the ration was about 25 ppm F and above, the differences were smaller between lots and the overlapping of individuals between lots was greater. These results indicate that urine analyses should be used only in conjunction with other criteria in diagnosing or determining the extent and severity of fluorosis. These results indicate that, for reliable information, it is advisable to secure samples from several animals in a group and, where possible, at periodic intervals.

The occurrence and degree of dental fluorosis is dependent upon the level and availability of fluorine ingested, period of time, age of animal, and amount of fluorine stored in the body. These results indicate the value and limitations of teeth as an aid in diagnosing bovine fluorosis.

Fluorine content of the rib biopsies, leg or jaw bones were related to level of fluorine ingestion and length of time the given levels were ingested. Analyses of fetal bones or of bones from calves at weaning time, that secured most of their nutrients from the cow's milk, show that increased F ingestion under conditions of Experiment I resulted in no appreciable increased transfer of fluorine to the fetus or to the calf through the cow's milk. Calves with their dams on pastures where the forage contained materially increased fluorine had a high content of fluorine in their bones at weaning time. This was due to the calves grazing the pastures as shown by comparing Lots 1, 3, and 4, with Lots 14, 15, and 16.

EXPERIMENT II—LOTS 20A–26

Objectives

The purpose of this experiment was to study the physiological changes in beef cows associated with the feeding of various levels of fluorine (0–50 ppm F) as sodium fluoride and to determine the effectiveness of aluminum sulfate in the alleviation of fluorotic symptoms. This experiment was designed in part, as a replication of Experiment I.

The several phases of this experiment include effects on:

1. Feed consumption and efficiency.
2. Growth, gain, and general physical conditions.

3. Reproduction of cows and calf production.
4. Digestion and mineral balance.
5. Bones, including amounts of fluorine stored in bones of cows and calves.
6. Various components of blood.
7. Urinary fluorine.
8. Teeth.

Experimental Procedure

The cattle used in this experiment were grade Hereford heifers purchased as yearlings in Texas and Tennessee in the summer of 1950. These heifers were born about January through April in 1949.

The heifers were free from tuberculosis and Bangs disease when shipped. Upon arrival at the Experiment Station, they were vaccinated with Prophylactic Brucella vaccine. Shortly after the heifers arrived from Texas, several of them were killed by lightning. Replacement heifers were purchased from a local herd.

The heifers were allotted, on the bases of type, grade, condition, source and weight, into 15 lots with three animals per lot, as shown in Table 16.

These lots, 20A through 24B, were fed individually in the barn, starting in October, 1950. All animals in these lots were on control rations for two weeks in order to adjust them to barn conditions. At the end of this period, the heifers were started on their respective rations.

The concentrate for Lots 20A through 24B was the same basic mixture fed to cows in Experiment I, with NaF and aluminum sulfate hydrate ($Al_2(SO_4)_3 \cdot 18\ H_2O$)[a] added in the manner described for NaF in Experiment I. General management of the animals and data collected were the same as those described for Experiment I.

In addition to the barn-fed groups, five lots, 25A through 26C, were started on pasture. These groups were added to Lots 16 and 14 respectively in Experiment I.

These heifers were placed on pasture in October, 1950, and were started on concentrate mixtures two weeks later. They were fed individually 1 pound of concentrate mixture daily until June, 1951. It was found that the heifers getting the concentrate containing $Al_2(SO_4)_3$ would not consume sufficient quantities to justify their continued feeding.

After June, 1951, the animals in Lots 25A through 26C were managed in the same way as those in Lots 16 and 14, respectively.

Lots 20A through 26C were bred to calve as two-years-olds in 1951 and were handled, during and after calving, the same as groups in Experiment I.

The same general procedures as to teeth pictures, weights, calving, feed sampling, etc., were followed in Experiment II as in Experiment I.

[a] Aluminum sulfate hydrate will be referred to as aluminum sulfate in the text.

TABLE 16.—PLAN OF EXPERIMENT II, LOTS 20A–26C

Lot no.	No. cows	F added in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Total F in ration ppm	Av. initial wt. Oct. 1950	Av. daily Mg. F/Kg. body wt.
20A	3	0	0	8	647	.18
20B	3	0	0.5	8	653	.18
21A	3	20	0	28	665	.60
21B	3	20	0.5	28	628	.67
22A	3	30	0	38	653	.83
22B	3[b]	30	0.5	38	694	.82
23A	3	40	0	48	660	1.05
23B	3	40	0.5	48	651	1.04
24A	3	50	0	58	656	1.20
24B	3	50	0.5	58	655	1.25
25A	3	Control Pasture	0		678	
25B	3	Control Pasture	0.5 [a]		621	
26A	3	B_2 Pasture	0		664	
26B	3[c]	B_2 Pasture	0.2 [a]		684	
26C	3	B_2 Pasture	0.5 [a]		675	

[a] When aluminum sulfate was added to the concentrates that were used to start cows on pasture, it was unpalatable and resulted in very little consumption. This phase on pasture was discontinued and animals in Lots 25 and 26 considered as only two groups. This was not true in the barn-fed groups, 20A–24B.
[b] Lost cow from calving.
[c] One cow was sacrificed from this lot to obtain data.

Results and Discussion

Feed Consumption. Results shown in Table 17 reveal that there probably was no appreciable difference in the feed consumption of Lots 20A, 21A, 22A and 24A for the period of October, 1950, to October, 1952.

In a comparison of the A groups (without aluminum sulfate added) with the B groups (with aluminum sulfate added), all B groups consumed slightly less concentrate and slightly more hay except Lot 23 in which A consumed more hay than B. An explanation for this probably is that the aluminum sulfate made the concentrate ration less palatable. This is further substantiated by a comparison of Lots 25A with 25B, and of 26A and C groups for the first feeding period.

The feed consumption of cows in Experiment II, having the same levels of fluorine intake as corresponding groups in Experiment I, was comparable for the first two years. In studying all the data, one should remember that the heifers in Experiment II were about six months older and weighed from 621 to 694 pounds per lot as compared to 453 to 523 pounds per lot in Experiment I. Furthermore, heifers in Experiment II had been on good pasture all summer in Texas and Tennessee, whereas heifers in Experiment I had been on dry winter feed and were wintered in average-to-thin condition prior to being started on test.

TABLE 17.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON FEED CONSUMPTION OF COWS IN EXPERIMENT II, LOTS 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Initial to Oct. 1951 Av. Daily Hay lbs.	Initial to Oct. 1951 Av. Daily Conc. lbs.	Oct. 1951 to Oct. 1952 Av. Daily Hay lbs.	Oct. 1951 to Oct. 1952 Av. Daily Conc. lbs.	Initial to Oct. 1952[a] Av. Daily Hay lbs.	Initial to Oct. 1952[a] Av. Daily Conc. lbs.
20A	8	0	15.17	3.11	15.48	3.98	15.32	3.54
20B	8	0.5	15.40	3.11	15.81	3.96	15.60	3.53
21A	28	0	14.30	3.11	14.87	3.97	14.58	3.54
21B	28	0.5	14.54	3.10	15.35	3.97	14.94	3.53
22A	38	0	13.92	3.11	15.16	3.95	14.53	3.52
22B	38	0.5	15.86	3.10	16.11	3.99	15.99	3.54
23A	48	0	15.81	3.10	16.12	3.99	15.96	3.54
23B	48	0.5	14.19	3.05	15.22	3.97	14.70	3.50
24A	58	0	13.86	3.11	14.12	3.99	13.99	3.54
24B	58	0.5	15.53	3.10	16.23	3.99	15.88	3.54

[a] No statistical significant difference between lots.

Cows in Experiment II are to be carried on experiment several years to indicate whether Lots 23 or 24 show results similar to those of Lots 5 and 6 in Experiment I.

Weights and Gains. There was no appreciable difference between the average daily gains or total gains and weights of any of the groups compared to the controls as shown in Table 18.

These results indicate that, under the conditions of this experiment and for the period reported, the addition of fluorine as NaF up to 50 ppm and/or aluminum sulfate as 0.5 percent of the ration had no appreciable effect on weights or gains.

Reproduction and Calf Records. Cows in these lots calved in 1951 and 1952. Table 19 gives the yearly and overall average of the number of calves born, average daily calf gain, and overall number of calves born and raised. There were no significant differences between lots in average daily calf gains. All calf weights were adjusted for years and sex differences to a comparable basis for comparison. The method used was one developed at this station for use in beef cattle breeding studies. It appears, also, that the number of calves born or raised in the various lots was not influenced by the rations the animals consumed. A summary of data on the cows in lots ingesting similar levels of fluorine, including Experiments I and II, indicates that an addition to 50 ppm fluorine as NaF does not affect calf production under the conditions of these experiments.

A summary of pasture Lots 14 and 26, and Lots 16 and 25, on number of calves born or raised indicates that, to date reported and under these conditions, there was no appreciable difference between these groups.

TABLE 18.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON WEIGHTS AND GAINS OF COWS IN EXPERIMENT II, LOTS 20A–26C

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Initial wt. Oct. 1950	Wt. Oct. 1951	Av. daily gain Initial to Oct. 1951	Wt. Oct. 1952	Av. daily gain Oct. 1951 to Oct. 1952	Av. daily gain Initial to Oct. 1952	Average gain of cows by number of calves raised 0	1	2
20A	8	0	647	879	.61	1020	.38	.50		.50(3) [a]	
20B	8	0.5	653	864	.56	1008	.39	.48			.48(3)
21A	28	0	665	852	.50	984	.36	.43		.43(2)	.42(1)
21B	28	0.5	628	781	.41	923	.38	.40			.40(3)
22A	38	0	653	838	.49	959	.33	.41	.43(1)	.35(1)	.45(1)
22B	38	0.5	693	849	.41	988	.38	.39			.39(2)
23A	48	0	660	867	.55	1080	.58	.56		.63(1)	.53(2)
23B	48	0.5	651	841	.50	940	.27	.39		.36(1)	.40(2)
24A	58	0	656	836	.48	1011	.48	.48	.66(1)	.53(1)	.23(1)
24B	58	0.5	655	942	.76	1037	.26	.51		.65(2)	.24(1)
25A and B	Control Pasture		650	937	.76	988	.14	.45		.45(5)	.45(1)
26A, B and C	B_2 Pasture		673	866	.51	1014	.40	.46	.58(1)	.63(1)	.41(6)

[a] Refers to number of cows.

TABLE 19.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON REPRODUCTION AND CALVES OF COWS IN EXPERIMENT II, LOTS 20A–26C

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	No. cows	1951 No. cows calving	1951 Calves raised No.	1951 Calves raised Av. daily gain	1952 No. cows calving	1952 Calves raised No.	1952 Calves raised Av. daily gain	1951–52 Total No. cows calving	1951–52 Total Calves raised No.	1951–52 Total Calves raised Av. daily gain [a]
20A	8	0	3	1	1	1.07	2	1	1.10	3	2	1.09
20B	8	0.5	3	3	2	1.40	3	3	1.40	6	5	1.40
21A	28	0	3	2	2	1.16	2	1	1.36	4	3	1.22
21B	28	0.5	3	3	2	1.29	3	2	1.34	6	4	1.32
22A	38	0	3	1	1	1.35	2	2	1.37	3	3	1.36
22B	38	0.5	2	2	2	1.52	2	2	1.33	4	4	1.42
23A	48	0	3	3	3	1.33	2	1	1.20	5	4	1.30
23B	48	0.5	3	2	2	1.22	3	3	1.06	5	5	1.13
24A	58	0	3	2	2	1.10	1	0	—	3	2	1.10
24B	58	0.5	3	1	1	1.40	3	3	1.38	4	4	1.38
25A and B	Control Pasture		6	1	1	1.80	6	6	1.76	7	7	1.76
26A, B and C	B_2 Pasture		8	7	7	1.69	6	5	1.71	13	12	1.70

[a] There was no statistical difference between the average daily calf gain of the controls and any other lot.

Table 20.—Effects of Fluorine and Fluorine Plus Aluminum Sulfate on Digestibility of Nutrients Fed Cows in Experiment II, Lots 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	No. animals	Dry matter intake gms.	Apparent Digestibility [a] Dry matter percent	Crude protein percent	Ether extract percent	Crude fiber percent	Nitrogen-free extract percent
20A	8	0	2	7912	63.4	65.5	47.6	51.4	74.0
20B	8	0.5	2	7300	63.6	63.6	56.3	50.6	74.7
21A	28	0	3	7010	64.2	62.5	47.5	54.4	73.3
21B	28	0.5	2	7176	62.8	58.4	49.4	52.8	72.3
22A	38	0	1	7609	62.9	65.4	62.8	56.5	71.2
22B	38	0.5	3	7609	62.2	61.6	55.0	52.6	72.0
23A	48	0	3	7136	62.9	62.4	54.1	58.2	73.7
23B	48	0.5	2	7355	65.1	61.4	59.0	59.0	74.1
24A	58	0	2	6574	66.2	61.7	50.8	58.2	75.2
24B	58	0.5	3	7807	64.5	64.7	56.6	54.9	74.6

[a] Trials conducted during 1952.

TABLE 21.—EFFECTS OF FLUORINE ON AVERAGE DAILY BALANCES OF FLUORINE, CALCIUM, PHOSPHORUS, AND NITROGEN OF COWS IN EXPERIMENT II, LOTS 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	No. animals	Urinary Fluorine [a]		Fecal F mg.	Daily Balance			
				F content ppm	Total F mg.		F mg.	Ca gm.	P gm.	N gm.
20A	8	0	2	3.0	27.8	27.4	1	−1.7	−.9	1.5
20B	8	0.5	2	2.0	16.2	27.1	13	−2.1	−4.6	1.0
21A	28	0	3	9.9	79.3	55.0	82	−1.6	−5.8	−6.5
21B	28	0.5	2	5.6	44.8	119.4	59	2.6	.3	4.7
22A	38	0	1	35.0	198.4	66.7	37	−2.8	.9	14.2
22B	38	0.5	3	7.1	54.2	159.4	111	−5.8	−.1	4.6
23A	48	0	3	14.2	109.0	71.5	235	−6.8	1.6	−5.2
23B	48	0.5	2	10.5	69.3	177.4	74	−2.2	−13.0	−0.4
24A	58	0	1	18.5	99.7	122.5	216	−6.4	.3	−10.6
24B	58	0.5	3	11.7	87.5	214.1	206	−1.0	−7.5	0.4

[a] Trials conducted during 1952.

Digestion and Balance Studies. Similar results are shown on the digestibility of nutrients as affected by feeding various levels of fluorine up to 50 ppm F added as NaF in Experiments I and II. There was no appreciable difference in the apparent digestibility of nutrients between lots.

The addition of aluminum sulfate to the rations of the cows, as shown in Table 20, appeared to have no appreciable effect upon the digestibility of the ration nutrients.

In the mineral balance studies reported in Table 21, the ppm fluorine in the urine of the cows on levels of fluorine ingestion at 20 ppm and above is different from those shown for Experiment I in Table 7. Similar differences have been suggested by comparing information in personal communications with other investigators. This is further evidence that urinary F values may be misleading and should be considered as only part of the criteria in diagnosing fluorosis. The amount of fluorine stored in the body is related, in general, to the amount of fluorine ingested, except that the addition of 0.5 percent aluminum sulfate to the ration will increase the amount of fluorine excreted in the feces, decrease amount excreted in the urine, and decrease body storage. Exceptions to this were found in comparing Lots 20A to B, and 22A to B.

The calcium, phosphorus and nitrogen balance figures probably show no appreciable differences between lots. The negative balance figures may be partially explained by reduced consumption by the cows when they were subjected to metabolism stanchion conditions.

Fluorine Content of Bones. Since these animals are being continued on experiment, chemical analysis for fluorine storage in the bone is limited to one rib analysis from each animal. The source of this analysis is the rib resections made in the summer of 1953. At that time the animals had been on the various rations for approximately 38 months. Results of these analyses are shown in Table 22. Within either series, with or without aluminum sulfate, the fluorine content of the rib biopsies is directly related to the level of fluorine ingestion.

As shown in this table, the fluorine content of the left twelfth rib was significantly reduced by the addition of 0.5 percent aluminum sulfate to rations that had 30, 40 and 50 ppm fluorine added (Lots 22B, 23B and 24B, respectively) when compared to rib analyses from animals fed comparable levels of fluorine with no aluminum sulfate added (Lots 22A, 23A and 24A, respectively). This reduction in fluorine storage was also evident at the 20 ppm level in the group receiving aluminum sulfate (Lot 21B) when compared to the group receiving the same level of fluorine without aluminum sulfate (Lot 21A). However, the amount of reduction in this case was not significant, as measured by the "T test" described by Snedecor (1946).

It can be noted that the average fluorine content of the ribs in Lot 24B

(50 ppm F + 0.5 percent $Al_2(SO_4)_3$ added) is slightly lower than the average fluorine content of the ribs in Lot 22A (30 ppm added). Also, the average for Lot 23B (40 ppm F + 0.5 percent $Al_2(SO_4)_3$ added) approximates the analyses of Lot 21A (20 ppm F added).

TABLE 22.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON RIB FLUORINE CONTENT OF COWS IN EXPERIMENT II, LOTS 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Animal no.	F content in ribs ppm	Av. F content in ribs ppm	F reduction ppm [a]	F reduction percent
20A	8	0	100	800	*667*	0	0
			101	600			
			102	600			
20B	8	0.5	103	600	*667*		
			104	700			
			105	700			
21A	28	0	106	2900	*2267*	467	20.6
			107	1800			
			108	2100			
21B	28	0.5	109	1800	*1800*		
			110	1500			
			111	2100			
22A	38	0	119	3200	*3267*	1167 [b]	35.7
			113	3000			
			114	3600			
22B	38	0.5	112	2100	*2100*		
			117	2100			
23A	48	0	118	3900	*4000*	1667 [c]	41.7
			115	3900			
			120	4200			
23B	48	0.5	121	2100	*2333*		
			125	2400			
			123	2500			
24A	58	0	124	4400	*4967*	1867 [b]	37.6
			122	5200			
			126	5300			
24B	58	0.5	127	2400	*3100*		
			128	3200			
			129	3700			

[a] Calculated reduction in F storage attributed to dietary $Al_2(SO_4)_3$.
[b] Statistically significant at .05 level.
[c] Statistically significant at .01 level.

Metacarpal and mandible samples were obtained from the 1951 calves, but only metacarpal samples were taken from the 1952 calves.

The 1951 calves received no feed other than their dams' milk. The 1952 calves were fed hay *ad lib.* and a limited amount of concentrate similar to the mixture fed the cows (no fluorine added).

The average analyses of these bones are shown in Table 23. Since there

TABLE 23.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON BONE FLUORINE CONTENT OF CALVES FROM COWS IN EXPERIMENT II, LOTS 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	1951 No. analyses	1951 Av. F in mandible ppm	1951 Av. F in metacarpal ppm	1952 No. analyses	1952 Av. F in metacarpal ppm
20A	8	0	1	110	120	1	134
20B	8	0.5	2	245	205	3	94
21A	28	0	2	230	210	1	167
21B	28	0.5	2	185	185	2	90
22A	38	0	1	250	220	2	140
22B	38	0.5	2	190	185	2	114
23A	48	0	3	193	217	1	152
23B	48	0.5	2	150	175	3	134
24A	58	0	2	150	185	0	—
24B	58	0.5	1	210	240	3	138

was a difference in analysis between years, these results are shown separately. The calves slaughtered in 1951 were an average of 55 days older than the 1952 calves.

It would appear from these analyses that the addition of fluorine and/or aluminum sulfate to the rations of the cows had no appreciable effect on the fluorine content of bones from calves produced by these cows under the conditions of this test. This further confirms similar results found in Experiment I.

Blood Studies. A study of the blood analysis data in Table 24 reveals that there was relatively little difference between any of the groups for the factors studied under conditions of this test. Although these data and blood data reported on Experiment I are limited, the results are similar and agree with other blood data in reference to the effect of increased fluorine ingestion on blood factors. High levels reported in Experiment III do show some differences.

Urinary Fluorine. Table 25 is presented to provide a comparison between a limited number of samples of a single voidation urine fluorine analysis, which was taken during the seven-day metabolism trial, with the urine fluorine analysis of the seven-day composite analysis. These data show the effect of aluminum sulfate as an alleviator by reducing the fluorine content

TABLE 24.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON BLOOD FROM COWS IN EXPERIMENT II, LOTS 20A–24B

				Whole Blood					Differentials					Plasma	Serum		
Lot no.	Total F in ration ppm	Allevi-ator added $Al_2(SO_4)_3$ percent	No. ani-mals	Hemo-globin (gms./ 100 ml.)	Hema-tocrit percent	Specific gravity	RBC (cmm.)	WBC (cmm.)	Eosino-philes percent	Baso-philes percent	Neutro-philes percent	Lympho-cytes percent	Mono-cytes percent	Specific gravity	Calcium (mgs./ 100 ml.)	Phos-phorus (mgs./ 100 ml.)	Mag-nesium (mgs./ 100 ml.)
20A	8	0	3	15.9	43.57	1.065	8,500,000	8,700	19	0	21	59	1	1.033	10.4	5.92	2.12 [a]
20B	8	0.5	3	13.1	36.58	1.060	6,737,000	10,267	15	0	24	60	1	1.032	10.2	6.31	2.14 [a]
21A	28	0	3	14.6	39.89	1.063	7,710,000	8,100	18	0	23	58	1	1.033	10.4	6.76	2.10 [a]
21B	28	0.5	3	15.4	40.82	1.064	8,458,000	8,100	16	0	23	60	1	1.032	10.5	5.75	2.34 [b]
22A	38	0	3	15.2	40.68	1.064	7,760,000	8,117	18	0	28	53	1	1.033	10.9	6.04	2.07 [a]
22B	38	0.5	2	15.8	41.28	1.064	8,775,000	7,000	14	0	20	66	0	1.033	10.3	4.78	2.43 [a]
23A	48	0	3	14.0	38.87	1.060	7,317,000	9,300	23	0	23	53	1	1.031	10.5	6.57	2.30 [b]
23B	48	0.5	3	14.8	40.59	1.064	7,780,000	8,067	9	0	39	51	1	1.033	11.0	6.37	2.34 [b]
24A	58	0	3	15.1	40.38	1.064	7,637,000	9,183	23 [a]	0 [a]	21 [a]	56 [a]	0 [a]	1.032	10.6	6.24	2.14 [b]
24B	58	0.5	3	13.8	36.99	1.061	6,883,000	8,400	21	0	25	53	1	1.032	10.6	7.10	2.39 [b]

[a] One animal.
[b] Average of 2 animals.

Table 25.—Effects of Fluorine and Fluorine Plus Aluminum Sulfate on Urine Fluorine Content of Cows in Experiment II, Lots 20A–24B

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Single Voidation No. Samples	Single Voidation Av. F content ppm [a]	Seven-Day No. Samples	Seven-Day Av. F content ppm [a]
20A	8	0	2	10.2	2	3.0
20B	8	0.5	3	3.3	2	2.0
21A	28	0	2	18.0	3	9.9
21B	28	0.5	2	5.8	2	5.6
22A	38	0	3	10.8	1	35.0
22B	38	0.5	4	6.0	3	7.1
23A	48	0	3	25.6	3	14.2
23B	48	0.5	3	—	2	10.5
24A	58	0	3	34.5	2	—
24B	58	0.5	3	11.6	3	11.7

[a] Standardized specific gravity to 1.040.

of the urine. Further, these show some of the wide variations in an individual sample as evidenced by the seven-day composite urine analysis from one animal in Lot 22A as compared to the single voidation analysis, and the single voidation analysis of Lot 20A compared to the seven-day analysis.

Detailed teeth readings were made monthly or bi-monthly which included detailed descriptions and colored photographs as discussed in Experiment I.

In order to present some data on the effect aluminum sulfate might have on reducing tooth lesions caused by excess levels of fluorine, Table 26 is presented as a record of the current classification of the incisors of all cattle in this experiment. This classification was made from the readings made in the latter part of 1952, and classifications made in 1953. The markings are usually bilateral, but in cases where this is not true, they are shown separately. Wear also was ascertained and is recorded for the individual teeth.

A study of the teeth classification of the control lot shows that all teeth, except those of one animal, classified 1A. Animal No. 102 in Lot 20A had staining on intermediates and laterals of incisors. The teeth of cows in Lots 21A and B (20 ppm F added) showed staining except in two animals, Nos. 109 and 110, which had caries in the corners and intermediates, respectively.

It should be pointed out that all these cows were born from January to May, 1949, and started on test in October, 1950. These cows were from 17 to 22 months of age. Since they were bought from grade cattle herds, the ages can be determined only insofar as the commercial producer knew

Table 26.—Classification of Incisor Teeth of Cows in Experiment II, Lots 20A–26C

Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Animal no.	1st Pair C[a]	2nd Pair C	3rd Pair C	4th Pair C	Lot no.	Total F in ration ppm	Alleviator added $Al_2(SO_4)_3$ percent	Animal no.	1st Pair C	2nd Pair C	3rd Pair C	4th Pair C
			100	1A	1A	1A	T				103	1A	1A	1A	T
20A	8	0	101	1A	1A	1A	L-T R-in 8mm	20B	8	0.5	104	1A	1A	1A	T
			102	1A	2	2	1B				105	1A	1A	1A	1A
			106	1A	2	L-4 R-[b]	T				109	1A	2	1B	2
21A	28	0	107	1A	2	2	T	21B	28	0.5	110	2	1B	2	T
			108	[c]	1B	2	T				111	1B	1BX	2	2
			113	1B	2	2	2				112	1A	1B	2	T
22A	38	0	114	1A	L-3 R-2	2	L-4 R-3	22B	38	0.5	117	1A	1A	2	T
			119	1A	2	2	2								
			118	1B	2	4	5A				121	1A	1A	2	T
23A	48	0	115	1A	2	5A	5A	23B	48	0.5	125	1A	1B	2	T
			120	1A	2	4	T				123	1A	1A	2	2
			122	1B	2	4	5A[b]				127	1A	2	4	T
24A	58	0	124	1A	2	4	4	24B	58	0.5	128	1A	2	2	2
			126	1B	3	5A	T				129	1A	L-3 R-2	2	T
			80	1A	1A	1A	1B[b]				83	1A	1A	1A	T
25A	Control Pasture		81	1A	1A	1A	1A	25B	Control Pasture		84	1A	1A	1A	1A[b]
			82	1A	1A	1A	1A				85	1A	1A	1B[b]	T
			90	1A	2	4	T				94	1A	2	4 to 5A	T
26A	B_2 Pasture		91	1A	2	4	T	26B	B_2 Pasture		95	1A	2	5A	4[b]
			92	1B	4	5A	T								
			96	1B	4	5B	T								
26C	B_2 Pasture		97	1A	R-1B L-2	5B	L-T R-5A[b]								
			98	1A	3	4[b]	T								

[a] C refers to Classification of tooth. See table 15.
[b] Early.
[c] Tooth mechanically broken out.

the approximate calving dates. A study of Table 26 reveals that the centrals of all cows came in reading 1A or B (normal) except for Cow 110 in Lot 21B and Cow 92 in pasture Lot 26A.

A close study of the data on aluminum sulfate fed (B) groups shows that the alleviator did decrease the teeth effects. This fact is most noticeable by comparing Lot 23A with Lot 23B. The teeth of cows in Lot 23B had no visible effects other than a 2 reading while Lot 23A had some teeth readings of 3, 4, and 5.

A comparison of the cows in this experiment on 20, 30, 40 and 50 ppm F added levels, and pasture groups 25 and 26 with similar lots in Experiment I, definitely shows that the age of the animal when first exposed to increased dietary fluorine, and stage of tooth development when first exposed, are very important factors in determining the effects on any particular tooth. This must be considered whenever incisors are to be considered in diagnosing fluorosis. These facts are shown whether comparing the barn-fed or pasture-fed groups.

This experiment, combined with other experiments, shows that the teeth are one good criterion to use with others in determining the extent of fluorine to which the animal was subjected during the time of development of a specific tooth.

Summary

The cows in Lots 20A through 26C, Experiment II, were started on test in October, 1950, as yearlings at approximately 17 to 22 months of age. Each F level was fed the control ration with and without aluminum sulfate added, plus fluorine added as NaF to give 20, 30, 40 and 50 ppm fluorine in the total ration. These lots without aluminum sulfate were a repetition of similar lots in Experiment I and were fed and managed as described for cows in Experiment I.

For the period reported, October, 1950, to October, 1952, there was no appreciable difference in feed consumption, weights and gains, reproduction and calf records, fluorine content of calf bones, digestibility of nutrients in the rations, and blood data which could be attributed to variation in levels of fluorine ingested. The fluorine content of rib, urinary fluorine content and teeth effects varied, in general, with the level of fluorine ingested. Results of rib biopsies indicated that the addition of aluminum sulfate as an alleviator resulted in a reduced bone fluorine content. This was also reflected in the increased fluorine excreted in the feces, the reduced excretion of fluorine in urine and the decreased damage of fluorine to incisors.

A comparison of the teeth classification (Tables 14 and 26) of cows on Experiment I with those on Experiment II, on similar level of fluorine ingestion, definitely shows that the age of the animal and stage of tooth devel-

opment at the time of increased fluorine ingestion are important factors in determining effects upon specific teeth.

EXPERIMENT III, LOTS 30–36

Objectives

After three years of feeding cows rations containing from 10 to 100 ppm of fluorine added as sodium fluoride, the Tennessee workers could find few of the reported "characteristic" symptoms such as black diarrhea, easily palpated exostoses, elongated hoofs, extreme emaciation, and long, rough hair coat which the literature generally associates with fluorosis in cattle. It was considered, therefore, that possible reasons for lack of these symptoms might include:

1. The reports in the literature might have been related to cattle which were receiving feed containing levels of fluorine higher than 100 ppm.
2. The diagnosis may have been based upon a correlation between current fluorine intake and reported symptoms, rather than the fluorine history of the animal. Consequently, the reported symptoms may have resulted from fluorine consumed from several months to several years previous to the observed diagnosis of fluorosis in the cattle. Furthermore, methods of chemical analysis for fluorine may have been continually improved, and early methods might have shown only a fraction of the actual fluorine in the feeds consumed.
3. There is a possibility that some of the symptoms reported in the early literature and attributed to fluorosis might have been secondary in nature or the result of abnormal conditions to which the animals had been previously subjected. It is known, for example, that an injury or fracture of a bone may result in a palpable exostosis. Such injuries could be broken ribs or other results of rough handling; or injury to legs as a result of cows' crawling over a solid structure. Animals going through narrow doors or rushed through doors or around corners may injure a hip bone, causing a condition that would indicate malformation of bony structures.
4. Management problems also might have caused uncertainty in the diagnosis. Elongation of hoofs has been listed as a symptom of fluorosis, but at this station this has not been found more prevalent in cows receiving added fluorine than in control cows. Many cattle standing constantly with little exercise, or exercising only on soft ground, may have elongated hoofs. Beef show cattle, unless carefully groomed, often have elongated hoofs. Likewise, an animal off feed or suffering from starvation may exhibit a rough, long hair coat such as that reported as a symptom of fluorosis.

The purpose of this phase of the investigation, therefore, was to study further the effects of feeding high levels of fluorine as sodium fluoride (100 to 1200 ppm added) on pregnant heifers.

The several phases of this experiment include effects of fluorine on:

1. Feed consumption.
2. Weights and gains.
3. Bones, including amounts of fluorine stored in bones of cows and calves.
4. Soft tissues.
5. Various components of blood.

Experimental Procedure

Fourteen grade Hereford heifers of known nutritional backgrounds, approximately 20 to 24 months of age, were divided into seven lots of two animals each on the bases of weights, condition, type and current feed consumption.

The daily ration consisted of four pounds of a concentrate mixture and a full feed of chopped, mixed legume hay. The concentrate was three parts of ground yellow corn and one part of cottonseed meal (41 percent protein). The control group (Lot 30) received this ration and each of the other lots received hay *ad lib.* and the same concentrate with fluorine added as indicated in Table 27.

Table 27.—Plan of Experiment III, Feeding High Levels of Fluorine, Lots 30–36

Lot no.	No. animals	F added in ration ppm	Total F in ration ppm	Av. initial wt. lbs.	Av. daily Mg. F/Kg. body wt.
30	2	0	7	719	.15
31	2	100	107	719	2.26
32	2	200	207	725	3.98
33	2	300	307	734	4.12
34 [a]	2	600	607	818 [b]	6.13
35 [a]	2	900	907	781 [b]	6.25
36 [a]	2	1200	1207	806 [b]	8.98

[a] Started on test March 4, 1952.
[b] Initial weight March 4, 1952.

The concentrate for each lot was similar to, or the same as, that used in Experiments I and II.

Prior to the beginning of the "High Level Experiment," the fourteen heifers were on normal concentrate and hay rations for a 100-day preliminary feeding period. This period was used to accustom the heifers to their quarters and to provide individual feed consumption records for later comparison and for use in allotting the heifers for the test.

All heifers were fed in individual stanchions. The concentrate mixture and hay was fed once a day.

In the dry-lot the heifers had access to salt and clean, fresh water. Individual daily records of feed consumption and feed refusals were kept on each heifer.

The feeding trial began January 22, 1952. After 18 days' feeding, it became apparent that the heifers receiving 600, 900 and 1200 ppm F in their rations were consuming less than one pound of concentrate and reduced amounts of hay per day. This method of administering sodium fluoride was discontinued because the heifers were not receiving the necessary level of fluorine. The heifers then were placed on normal rations, and it was 24 days before they were again on feed. At this time, therefore, in an effort to insure the ingestion of the required ppm of fluorine, the calculated sodium fluoride was administered daily to each animal. The fluoride was given orally in a gelatin capsule. However, after two feedings, all heifers except one refused to consume their concentrates. Hay consumption dropped materially to about two to six pounds daily. Since individual consumption varied from day to day, it was necessary to calculate daily the amount of sodium fluoride to be administered, based upon feed records of the previous day.

Daily observations were made for any occurring abnormalities. The heifers were weighed periodically. At periodic intervals, blood samples were taken in order to determine blood calcium, phosphorus, hemoglobin, hematocrit, specific gravity and blood phosphatase. Teeth readings and pictures were made periodically.

General appearance and locomotion of the higher fluorine lots of heifers were recorded by color moving pictures which were taken at 37, 76 and 200 days after the capsule feeding of sodium fluoride was started. When an individual heifer became too weak to rise and continued in a recumbent position for two or more days, she was sacrificed for autopsy and tissue sampling. After about 14 months the experiment was terminated. The remaining cattle were slaughtered and autopsies made for comparison of the controls with the animals receiving 100, 200 and 300 ppm fluorine, respectively.

Results and Discussion

Feed Consumption. A study of Table 28 reveals that there was no appreciable difference between the feed consumption of Lot 30 and Lot 31. There was some decrease in feed consumption of Lot 32 (200 ppm F added) and a progressive, material decrease by Lot 33 (300 ppm F added). Compared to the foregoing lots, Lots 34, 35 and 36 practically quit eating concentrates within three days after receiving fluorine by capsule and decreased hay consumption by more than 50 percent. Lots 34, 35 and 36 practically starved to death as shown by feed consumption and weight losses. Lot 33 cows, after fluorine was administered by capsule, lost weight and were on a starvation consumption.

Table 28.—Effects of Fluorine on Feed Consumption and Weights and Gains of Cows in Experiment III, Lots 30–36

Lot no.	Total F in ration ppm		Jan. 22 1952	Mar. 4 1952	Apr. 9 1952	May 16 1952	June 3 1952	June 28 1952	July 25 1952	Sept. 4 1952	Nov. 28 1952	Feb. 19 1953	Mar. 17 1953	Av. for period: Gain	Av. daily gain	Hay	Conc.	Mg. F/Kg.
30	7	Av. wt., lbs.	719	828	872		878	882		972	982	960	924	206	.49			
		Av. lbs. per animal per day consumed:																
		Hay		14.1	14.3		13.7	11.2		12.0	15.2	15.4	15.1			14.1		
		Conc.		4.0	4.0		4.0	4.0		4.0	4.0	4.0	4.0				4.0	
		Mg. F/Kg. body wt.		.1859	.1778		.1731	.1582		.1353	.1159	.1353	.1555					0.16
31	107	Av. wt., lbs.	719	826	856		846	815		822	835	920	894	174	.41			
		Av. lbs. per animal per day consumed:																
		Hay		13.7	14.5		13.7	10.1		12.3	15.2	14.2	15.2			13.8		
		Conc.		4.0	4.0		4.0	4.0		4.0	4.0	4.0	4.0				4.0	
		Mg. F/Kg. body wt.		2.315	2.266		2.288	2.353		2.331	2.272	2.076	2.156					2.26
32	207	Av. wt., lbs.	725	820	852		848	782		706	638	780	766	41	.10			
		Av. lbs. per animal per day consumed:																
		Hay		12.5	14.1		15.1	10.0		9.7	13.5	12.2	14.1			12.6		
		Conc.		3.9	3.6		3.1	3.3		2.7	3.3	4.0	4.0				3.4	
		Mg. F/Kg. body wt.		4.153	3.784		3.288	3.698		3.396	4.456	4.477	4.585					3.98
33	307	Av. wt., lbs.	734	800	818		774	745 [a]		645	565	530	503	−231	−.55			
		Av. lbs. per animal per day consumed:																
		Hay		13.6	14.2		15.0	13.5 [a]		8.7	9.0	6.3	4.8			10.1		
		Conc.		1.9	1.9		1.4	1.7 [a]		1.6	1.4	3.9	4.0				2.2	
		Mg. F/Kg. body wt.		3.203	3.011		2.356	2.990 [a]		4.733	5.546	5.898	5.230					4.12
34	607	Av. wt., lbs.	729	818	683		536	512	430					−348	−2.66			
		Av. lbs. per animal per day consumed:																
		Hay			6.6		5.5	5.5	0.9							5.6		
		Conc.			0.8		0.2	0.2	0.6								.54	
		Mg. F/Kg. body wt.			6.440		6.316	5.134	6.636									6.13
35	907	Av. wt., lbs.	708	781	602	538	475 [b]							−274	−3.31			
		Av. lbs. per animal per day consumed:																
		Hay			3.8	3.8	1.9									3.2		
		Conc.			0.3	0.3	0.2										.29	
		Mg. F/Kg. body wt.			6.655	7.670	4.431											6.24
36	1207	Av. wt., lbs.	730	806	588	377 [c]								−316	−5.32			
		Av. lbs. per animal per day consumed:																
		Hay			3.2	3.4										3.2		
		Conc.			0.4	0.1											.31	
		Mg. F/Kg. body wt.			7.676	10.295												8.98

[a] Cows in Lot 33 were given fluorine by capsule from July, 1952.
[b] 6–5–52
[c] 5–24–52

There is no apparent explanation for the increased concentrate consumption of Lots 32 and 33 in November and December except the possibility that cooler weather was a factor. These data reveal that the level of fluorine which affected feed consumption in this test covering 422 days, was somewhere between 100 and 200 ppm.

Weights and Gains. The weights and gains or losses are shown in Table 28. Immediate loss of body weight resulted from administration by capsule, 600, 900 and 1200 ppm fluorine in the ration, as NaF. As soon as Lot 33 started receiving fluorine as NaF by capsule rather than in the concentrate, which made a change from about 3 to 5 mg./kg. of body weight, the cows consistently began losing weight. Under conditions of this test, Experiment III, whenever cattle were actually receiving 300 ppm or about 5 mg./kg. body weight there was a definite loss of weight. The cows in Lot 32 (200 ppm F) were significantly below Lots 30 and 31 in rate of average daily gain.

Fluorine Content of Bones. A study of Lots 30–33 shows that fluorine depositions in the bones were directly related to the level of fluorine ingestion. However, a comparison of Lots 34, 35 and 36, groups on 600, 900 and 1200 ppm F, shows that fluorine storage is affected not only by level ingested but also by the length of time the cattle consumed these levels of fluorine. This is shown in Table 29.

A comparison of the fluorine content of the leg bones with the fluorine content of jaw and ribs reveals that on a continuous level of intake for the same time, in general, the rib is highest in storage of F, next the jaw and then the leg. In general, this picture occurs when an increased level of fluorine is being fed continuously.

A study of fluorine content of bones from calves reveals that with cows receiving up to 300 ppm F, the data agree with Experiments I and II showing no appreciable increase in bone fluorine content of calves. However, the limited data in Lots 34, 35 and 36 indicate that at levels of 600 ppm fluorine and above there may be an appreciable transfer of fluorine from the dam to the fetus. More data are needed before reliable conclusions can be drawn.

Bone and Soft Tissue Changes. Bone lesions described here are considered to be due to the intake of fluorine over the various periods of time and at the different levels of concentrations used in this experiment. The responsibility of fluorine intake for the development of these bone lesions is established by the following:

1. The lesions were not present in the living animals at the start of the experiment.

2. The severity of the lesions at the end of the experiment showed dependency upon level of fluorine intake and total time during which fluorine was consumed.

TABLE 29.—EFFECTS OF HIGH LEVELS OF FLUORINE ON BONE FLUORINE CONTENT OF COWS AND CALVES IN EXPERIMENT III, LOTS 30–36

					Cow Bones								Calf Bones					Fetal Bones		
Lot no.	Total F in ration ppm	Animal no.	No. days on test	Av. no. days on test	F content in mandible ppm	Av. F content in mandible ppm	F content in metacarpal ppm	Av. F content in metacarpal ppm	F content in rt. 9 rib ppm	Av. F content in rt. 9 rib ppm	F content in rt. 10 rib ppm	Av. F content in rt. 10 rib ppm	F content in metacarpal ppm	Av. F content in metacarpal ppm	F content in mandible ppm	F content in rib ppm	Age in days	F content in metacarpal ppm	F content in rib ppm	Stage of development
30	7	−3	422		1200		1100		1400		1500		330				133			
		−6	422		1300		1500		1400		1300							500		—
				422		*1250*		*1300*		*1400*		*1400*		*330*						
31	107	−9	422		8800		5500		10300		9800		250				126			
		−10	422		10000		7500		11600		11200		190				182			
				422		*9400*		*6500*		*10950*		*10500*		*220*						
32	207	−1	423		12500		11600		12900		12000		270				166			
		−5	423		12600		10700		14800		14400		330				170			
				423		*12550*		*11150*		*13850*		*13200*		*300*						
33	307	−7	424		16400		14400		17900		17700		320				172			
		−14	424		13000		8700		14200		13800		290				171			
				424 [a]		*14700*		*11550*		*16050*		*15750*		*305*						
34	607	−4	117		9900		8300		10200		10300		1200		770		45			
		−8	142		10150		5700		10600		10000									
				130 [b]		*10025*		*7000*		*10400*		*10150*		*1200*						
35	907	−12	71		8500		6200		10400		9900							2200	2400	8–9 mos.
		−2	104		8400		4900		8900		8900		650		460	640	6			
				88 [b]		*8450*		*5550*		*9650*		*9400*		*650*						
36	1207	−11	40		6300		3500		8000		9200							960	960	8 mos.
		−13	81		8000		6400		9300		8700							Fetus too small for individual bone analysis.		
				60 [b]		*7150*		*4950*		*8650*		*8950*								

[a] These heifers received fluorine by capsule for the last 236 days.
[b] These heifers were given fluorine as NaF by capsule.

3. No similar lesions occurred in the control lot even though these animals were under conditions identical with the other lots except that no fluorine was added to their rations.

4. Similar lesions have been recorded in the literature and attributed to fluorine.

Following post mortem examination of animals in this experiment, two types of gross bone changes due to the consumption of fluorine as sodium fluoride were recognized. One type was a typical exostosis found on the external surfaces of the bones of the limbs, and on the mandible. The second type of bone change encountered was the even and regular thickening of the shafts of certain bones of the limbs and the rami of the mandibles. These thickened areas caused a general increase in the diameter of the affected bones. These bony thickenings resulted from proliferation of the periosteum chiefly on shafts of long bones, with the subsequent build-up of bone layers above the normal limits.

It could not be determined grossly whether there was much definite build-up of abnormal bone layers in the medullary cavity. Some evidence of decrease in the size of the medullary cavity was noted in mandibles which showed general thickening. In general, there was no definite pattern of changes in the medullary cavities of the long bones. Table 30 shows the occurrence of both exostoses and generalized bony proliferation in bones of heifers from each of the lots.

The exostoses encountered in post mortem examinations varied in size. The smallest were less than the size of a common pea (2 x 2 x 2 millimeters). The largest exostoses produced in this experiment were approximately the size of a small egg (5.5 x 4 x 3 centimeters). In most cases the exostoses did not rise more than 4 or 5 millimeters above the surface of the surrounding bone. Often many of these small exostoses merged, forming bony ridges several millimeters long. An example of such a ridge appeared on the lateral margin of the shaft of the radius of Cow −1 (200 ppm F added).

These typical exostoses were found, in some degree, on bones of animals from all lots except the controls. The metatarsi and metacarpi were most productive of exostoses. The scapula was least often affected. Bones on which exostoses were discovered are listed in order of the approximate frequency with which exostoses were found on them:

1. Metatarsus and metacarpus
2. Mandible
3. Tibia
4. Fibular tarsal
5. Radius and ulna
6. Femur
7. Pelvis
8. Humerus
9. Frontal bone
10. Maxilla
11. Scapula

Not all exostoses found on post mortem examination would have been detected by palpation on the living animal. The size of the exostosis and its

Table 30.—Effects of High Levels of Fluorine on Gross Bone Involvement of Cows in Experiment III, Lots 30–36

Lot no.	Total F in ration ppm	Animal no.	No. days ingestion	Frontal	Maxilla	Mandible	Ribs	Scapula	Hu-merus	Radius & Ulna	Meta-carpus	Bony Pelvis	Femur	Tibia	Fibular Tarsal	Meta-tarsus
30	7	−3	422	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL
		−6	422	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL
31	107	−9	422	NVL	NVL	T-S	NVL	NVL	NVL	NVL	T	NVL	NVL	NVL	NVL	T-S
		−10	422	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	NVL	T-S
32	207	−1	423	NVL	NVL	S-M	T [a]	NVL	NVL	S	Ex	NVL	NVL	S	M	Ex
		−5	423	NVL	NVL	S-M	NVL	NVL	NVL	NVL	M-H	NVL	T	NVL	M	M-H
33	307	−7	424	NVL	NVL	S	NVL	NVL	NVL	NVL	M	NVL	S	NVL	NVL	S
		−14	424	NVL	NVL	M	NVL	NVL	NVL	NVL	S	T	NVL	T	NVL	S-M
34	607	−4	117	NVL	NVL	S-M	NVL	T	NVL	T	Ex	S	T	T	S	Ex
		−8	142	T	NVL	S	NVL	NVL	T	T-S	M	S	NVL	S	S	H
35	907	−2	104	T	NVL	S	NVL	NVL	T	H [c]	Ex	T	T-S	M	S-M	Ex
		−12	71	T	T-S	S	T [b]	S	NVL	M	Ex	S	S	H	S-M	Ex
36	1207	−11	40	NVL	NVL	NVL	NVL	NVL	T	NVL	NVL	NVL	NVL	NVL		S
		−13	81	S-M	S-M	H	T [a]	NVL	S	T-S	Ex	NVL	NVL	M		Ex

[a] Not palpable.
[b] Only on 3 pairs. Not palpable.
[c] On Ulna only.

Legend
NVL—No visible lesions.
T—Traces, less than ⅛ of bone involved.
S—Slight, ⅛ to ¼ of bone involved.
M—Medium, ¼ to ½ of bone involved.
H—Heavy, ½ to ¾ of bone involved.
Ex—Excessive, ¾ or more of bone involved.

location determine whether it is palpable. Those on the scapula, humerus, tibia and tibial tarsus were not palpable in the living animal. Exostoses on the metatarsi and metacarpi probably could have been palpated in the living animal if they had reached a height of 5 millimeters or more above the surrounding bone and were located on the dorsal (front) surface or sides of the bone shaft. Exostoses of the mandible would have been accurately palpable in only a few cases.

In this experiment, post-mortem examination did not reveal exostoses on the articular surfaces of any of the bones. In a few cases (femur, scapula and radius of −12 and the femur of −2) exostoses were found on the margins of articular areas. In general, these apparently were not located in a position that interfered with articulation.

The formation of layers of bone, over what would have been the normal external limits of that bone, was observed chiefly in the metacarpus, metatarsus and mandible. The most marked example of bone overgrowth was found in the two animals receiving 200 ppm of added fluorine. On the enlarged metatarsal and metacarpal bones of one of these animals, the vascular grooves had been completely filled by new bone.

Gross Bone Changes by Lot: Lot 30—control ration: No gross exostoses or thickening of bone shafts were found. Both animals were on experiment 422 days.

Lot 31—100 ppm F added: One animal (−9) showed slight thickening of the rami of the mandible. This same animal showed slight irregularities on the volar surfaces of the metacarpi. Both animals exhibited slight, irregular swellings on the volar and lateral surfaces of the metatarsi. Both were on experimental rations 422 days.

Lot 32—200 ppm F added: Both animals showed slight to medium thickening of rami of both mandibles. There was a ridge of exostoses on each radius of animal, −1 measuring 20 x 18 mm. and rising 2 mm. above the bone surface (20 x 18 x 2 mm.). Metacarpi of both animals were enlarged. The vascular groove of the metacarpus was obliterated on animal −1. In addition to bony swellings, the metacarpi had large exostoses, each with two lesions, −1 measuring 5.5 x 4 x 3 cm. The left femur of −5 had a small area of irregular bony enlargement near the trochlea. Large exostoses (45 x 25 x 10 mm. and 15 x 10 x 3 mm.) were found on tibia of −1. The fibular tarsal bones of both animals were thickened. Metatarsi of both animals were thickened and irregular and the vascular grooves were filled. The ribs of −1 showed traces of bony swellings which could not have been palpated in the living animal. Both animals were on experimental rations 423 days.

Lot 33—300 ppm F added: Bony changes in this lot were no more extensive than in Lot 32 (200 ppm). Horizontal rami of mandibles showed medium, uneven swellings. Metacarpi were less involved than in Lot 32, with slight to medium bony swellings and no exostoses larger than 2 x 7 x 2 mm. This was the lowest level showing gross changes of the pelvis, with −14 having three exostoses, measuring 15 x 3 mm. and rising 5 mm. above the bone surface, on the medial face of the ischium near the obturator foramen. There was one small exostosis on each tibia of −14. Metatarsi showed less involvement than was shown in Lot 32. Metatarsi of −14 showed generalized thickening of the shaft plus a few exostoses, while metatarsi of −7 showed only general thickening. Both animals were on experiment 424 days.

Lot 34—600 ppm F added: This was the lowest fluorine level at which changes were found in the frontal bone, scapula and humerus. Small irregular exostoses appeared medial to orbits on frontal bones of −8. Mandibles showed slight enlargement. Several 1 x 1 x 1 mm. exostoses were found near the medial margin of the glenoid cavity of the scapula of −4. The articular surface was not involved. On the humerus of −8, there was one small (3 x 3 x 2 mm.) exostosis near the medial condyle and slight enlargement of the margins of the olecranon fosca. There were a few exostoses on the olecranon and the radius of each animal. The metacarpus of −4 showed excessive bony thickening of all surfaces. The metacarpus of −8 showed thickening and exostoses on the dorsal surface. The bony pelvis of each animal showed a slight, multiple, small exostosis on the ischium and ilium but none on the pubis. The right femur of −4 had one exostotic ridge 20 mm. long and 1 mm. high. Fibular tarsal bones of each animal showed multiple, small (1 x 1 x 1 mm.) exostoses. The metatarsi of each animal showed heavy to excessive involvement with multiple large exostoses. Animal −4 was on experimental rations 117 days, and −8 was on the rations 142 days.

Lot 35—900 ppm F added: One 8 x 15 x 2 mm. exostosis was found on the left frontal bone of −2 and two large (approximately 30 x 25 x 12 mm.) exostoses were found on the frontal bones of −12. This was the lowest level at which exostoses were found on the maxilla. Maxillae from both sides of −12 had a total of three exostoses, one measuring 20 x 15 x 4 mm. and two being smaller. Mandibles of both animals showed slight thickening. Three pairs of ribs on −12 had traces of bony swellings, none of which could have been palpated in the living animal. Several exostoses were found near the glenoid cavities of the scapulae of −12. The humerus of −2 had small exostoses on the margin of the medial condyle, and on the right only, one 18 x 5 x 1 mm. exostosis on the base of the lateral tuberosity. The radii of −2 had no visible lesions, but the ulnas showed thickening and large exostoses at the olecranons. Radii of −12 had multiple 1 x 1 x 1 mm. exostoses on the upper part of the dorsal surface of the shaft and the ulnas showed slight exostoses laterally. There was excessive bony enlargement, and there were exostoses of metacarpi of both animals. All three bones of the bony pelvis of −12 and only the ilia of −2 showed multiple, small exostoses. Femurs of both animals showed multiple, slight exostoses at the margins of the condyles and on the shafts. Tibiae of both animals had multiple exostoses on the shafts ranging in size from 3 x 3 x 1 mm. to 50 x 20 x 15 mm. Fibular tarsal bones of both animals appeared thickened. Metatarsi of both animals showed excessive involvement of all aspects except the articular surfaces. The vascular grooves of the metatarsi were partly obliterated. Animal −12 was on the experimental ration 71 days and −2 for 104 days.

Lot 36—1200 ppm F added: As indicated in Table 31, animal −11 of this lot showed very little bone change, while −13 showed extensive changes. Multiple exostoses appeared on the maxillae of −13 only. The mandible of −13 showed only heavy bony enlargement and exostoses. A few ribs of −13 showed only slight bony thickening which could not have been palpated in the living animal. One exostosis was found on the shaft of each humerus of −11 and multiple exostoses were found on the humeri of −13. There was slight enlargement of the radii of −13. Animal −11 showed no involvement of the metacarpi, while the metacarpi of −13 had excessive, generalized exostoses and enlargement. There were no visible lesions on the tibiae of −11, and only medium exostoses on those of −13. The metatarsi of −11 showed slight, localized, small (5 x 5 x 1 mm.) exostoses on the medial and dorsal margins. The metatarsi of −13 were found to have extensive exostoses and enlargements. Animal −11 was on the experimental ration 40 days, and − 13 for 81 days.

Microscopic bone changes were confined to the shafts of the long bones, rami of the mandibles, and the frontal and maxillary bones. Sections were not taken from the pelvis, in this experiment, for routine microscopic study. As was true in the gross study of the bones, articular surfaces did not appear involved under histological study.

The principal histological bone changes were proliferative. The osteoblasts became increasingly active and organic intracellular material was laid down in concentric layers over the normal limits of the older bone. At the same time, resorption was increased in the area of new bone to the extent that the resulting bone formation appeared, microscopically, to be very porous.

Gross examination of the soft tissues during autopsy did not show significant changes.

The significant changes in the microscopic structures of the soft tissues were found in the kidneys of the animals on the 900 and 1200 ppm fluorine levels and in the liver of one animal (−11) on the 1200 ppm level. In the kidneys from the lots on 900 and 1200 ppm fluorine, there were distinctive changes in the cells of the distal part of the convoluted tubules. These cells had a marked vacuolated effect and were nearly devoid of cytoplasm. The nucleus appeared pushed against the basement membrane, but the cell membrane did not seem to be distended.

In Lot 36, which received fluorine at the rate of 1200 ppm, animal number −11 showed changes in the hepatic cells which apparently were identical with the changes described in the distal convoluted tubules of animals from Lots 36 and 35 (900 ppm). Here again the cytoplasm of the cell was nearly non-existent. Wherever cells affected in this way were found, it was noted that practically all cells of the lobule were similarly affected. An accurate estimate of the number of liver lobules with vacuolated cells could not be made. In some sections, however, as high as 15 percent of the cells appeared to be affected.

Although kidney lesions were found in all four animals in the 900 and 1200 ppm lots, the liver lesions were noted only in animal −11, which received fluorine at the rate of 1200 ppm. It is also of interest to note that this cow lived 40 days on experimental rations, as compared to 81 days for the other animal in this same group and 71 and 104 days, respectively, for the two cows on the ration with 900 ppm of fluorine added. No clinical tests of liver function were run on these animals prior to their deaths.

Blood Studies. In the later stages of Experiment III, lots receiving 300 and 600 ppm of fluorine showed drops in hemoglobin and hematocrit levels in whole blood. There were also slight drops of both hemoglobin and hematocrit in animals receiving 100 and 200 ppm of fluorine added during the experiment, but these levels returned to normal as they neared the end of the experiment. Serum calcium and phosphorus appeared normal to slightly lower than normal in animals receiving 600, 900 and 1200 ppm fluorine. These lowered hemoglobin, hematocrit, calcium and phosphorus levels may have been due to lowered nutrient intake which would be an indirect rather than direct effect of high levels of F.

Summary

Effects of various high levels (control, 100, 200, 300, 600, 900 and 1200 ppm F added in total dry ration) of fluorine fed as sodium fluoride were studied with fourteen bred Hereford heifers, with two animals per lot. Under the conditions of this experiment, the following results were indicated:

1. Impaired appetite was one of the first indications of the abnormal effects resulting from ingestion of high levels of fluorine.

2. Cows ingesting 200 ppm F or more showed a decreased feed consumption. A direct relationship was found between the ingestion of fluorine at levels of 100 to 1200 ppm in the dry ration, and impaired appetite (considering period fed), weight loss and general unthrifty physical condition.

3. There was decreased weight gains in the lots receiving 200 ppm F compared to the control animals. The lots receiving 300 ppm and above lost weight.

TABLE 31.—EFFECT OF HIGH LEVELS OF FLUORINE ON BLOOD FROM COWS IN EXPERIMENT III, LOTS 30–36

Lot no.	Total F in ration ppm	No. animals	Apr. 1952	June 1952	July 1952	Aug. 1952	Jan. 1953	Feb. 1953
			Grams of Hemoglobin/100 ml.					
30	7	2	14.2	12.6	12.8	12.2	13.6	13.6
31	107	2	11.6	10.9	10.2	9.6	12.0	13.0
32	207	2	11.4	11.2	9.6	8.8	12.3	12.6
33	307	2	11.6	9.0	9.6	8.7	8.2	7.4
34	607	2	11.2	7.4	5.2 [a]	—	—	—
35	907	2	11.7	—	—	—	—	—
36	1207	2	10.2	—	—	—	—	—
			Percent Hematocrit					
30	7	2	42.4	38.3	36.7	35.7		38.2
31	107	2	34.6	31.4	28.8	27.8		34.9
32	207	2	34.4	34.4	28.5	27.0		35.4
33	307	2	34.7	32.4	29.0	25.7		21.3
34	607	2	35.8	28.0	17.2 [a]	—		—
35	907	2	35.2	—	—	—		—
36	1207	2	33.3					
			Specific Gravity Whole Blood					
30	7	2		1.056	1.056		1.057	1.058
31	107	2		1.051	1.050		1.056	1.056
32	207	2		1.052	1.050		1.056	1.057
33	307	2		1.050	1.050		1.046	1.028
34	607	2		1.044	1.040 [a]		—	—
35	907	2		—	—		—	—
36	1207	2		—	—		—	—
			Specific Gravity Plasma					
30	7	2	1.029	1.028	1.029		1.028	1.030
31	107	2	1.028	1.029	1.029		1.029	1.030
32	207	2	1.028	1.030	1.029		1.028	1.030
33	307	2	1.028	1.028	1.029		1.028	1.030
34	607	2	1.028	1.026	1.025 [a]		—	—
35	907	2	1.030	—	—		—	—
36	1207	2	1.028	—	—		—	—
			Serum Calcium					
30	7	2	10.4	10.0	9.3			
31	107	2	9.6	9.9	8.4			
32	207	2	10.4	9.1	8.2			
33	307	2	10.2	9.7	8.7 [a]			
34	607	2	8.8	8.6	—			
35	907	2	8.8	—	—			
36	1207	2	8.2	—	—			
			Serum Phosphorus					
30	7	2	7.25	8.96	8.02			
31	107	2	7.08	9.46	7.40			
32	207	2	7.13	9.12	7.38			
33	307	2	6.44	8.38	7.25			
34	607	2	6.22	8.08	5.12 [a]			
35	907	2	7.54	—	—			
36	1207	2	6.37	—	—			

[a] One animal.

4. Loose bowels occurred at various times in the animals receiving F levels of 300 ppm and higher, but this disorder did not resemble "black diarrhea," reported by Roholm (1937). This condition probably was due to a major decrease in feed consumption, which resulted in a large proportion of water and small amount of dry matter in the feces.

5. Fluorine stored in the bones was directly related to the amount of fluorine ingested and the length of time of ingestion.

6. Various degrees of stiffness and locomotive difficulties were observed in cows ingesting 200 ppm F and above.

Exostoses and bony enlargements were found upon post-mortem examinations of heifers which had been on fluorine levels of 100 ppm and above. The metatarsi and metacarpi appeared most sensitive of all bones to the effects of fluorine. Many of the bone changes found on autopsy would not have been palpable in the living animals.

Microscopic bone changes consisted of periosteal proliferation and evidence of increased resorption.

The kidneys of animals on 900 and 1200 ppm F levels showed nephrosis characterized by a vacuole-like appearance of cells in the distal convoluted tubule. The liver of one animal on 1200 ppm added fluorine, showed similar cellular changes.

Sheep Experiments

EXPERIMENT X, LOTS 1–9

Objectives

The purpose of this experiment was to study the several physiological and pathological changes associated with the feeding of various levels of fluorine to growing lambs. Also, a study was made of the alleviating effects of aluminum sulfate hydrate and aluminum chloride hydrate fed to growing lambs receiving 100 parts per million fluorine in their ration. The several phases of the experiment included the effects on:

1. Efficiency of feed utilization.
2. Weight gains.
3. The ability to digest crude fiber, crude protein, ether extract, and nitrogen-free extract.
4. Fluorine, calcium, phosphorus and nitrogen balances.
5. Blood levels of phosphorus, sodium, potassium, magnesium, and calcium as a function of time.
6. Quantitative fluorine storage in bone, heart, liver, kidney, and spleen.
7. Pathological changes in bone, heart, liver, kidney, and spleen.
8. The correlation of fluorine content of urine and dietary fluorine intake as a possible criterion for the diagnostic measurement of fluorosis in animals under field conditions.
9. Certain micro-organisms in the rumen.

Experimental Procedure

Seventy-five crossbred feeder wether lambs 8 to 12 months of age, weighing an average of 88 pounds, were used in this study. These animals were fed for approximately a two-month pre-experimental period to treat them for internal parasites, get them on feed, and accustom them to individual feeding facilities. All animals were docked and treated routinely with phenothiazine. During this period, the daily ration of chopped lespedeza hay and a concentrate mixture (80 percent ground yellow corn and 20 percent wheat bran) was gradually increased until each animal was consuming approximately one pound of hay and one pound of concentrate per day. They had free access to salt and water. Individual feed records were initiated on all animals within the last four weeks of this period. The animals were fed in the early morning and late evening, with one-half pound of concentrate provided at each feeding.

Twelve of these lambs, selected at random, were placed in metabolism stalls for a three-week preliminary training period. During this three-week period, final changes and adjustments were made in the metabolism stalls to standardize the balance procedure which was used throughout the experiment.

Following this preliminary period, seven-day collections were made on twelve lambs to determine the digestibility of feeds and mineral balances on representative animals to be used as the control. Representative samples of hay, concentrate, urine, and feces were taken daily from each animal, from which an individual composite sample for the seven-day collection period was analyzed for fluorine, calcium, phosphorus, crude protein, crude fiber, ether extract, and nitrogen-free extract.

Three animals, selected at random from the 75 lambs, were sacrificed one day before the experiment started. Tissue samples were taken for fluorine analysis and pathological studies. Results from these studies were used as base controls for all lots.

The remaining 72 feeder wether lambs were weighed, paint-branded, and divided into six lots of ten each and three lots of four each, using the criteria of weights, previous feed consumption, and general appearance. The six lots (Lots I through VI) of ten animals each were assigned treatments at random and the three lots (Lots VII through IX) of four animals each were assigned treatments at random within each group of lots, as shown in Table 32.

TABLE 32.—PLAN OF EXPERIMENT X—SHEEP, LOTS 1–9

Lot no.	F added in ration ppm	Alleviator percent	Total F in ration ppm	No. animals	Av. initial wt. lbs.	Initial wt. range lbs.
I	0		6	10	88.4	81–99
II	25		31	10	88.3	75–96
III	50		56	10	88.1	75–113
IV	75		81	10	88.2	76–96
V	100		106	10	88.3	77–104
VI	200		206	10	88.3	76–106
VII	100	0.1% $Al_2(SO_4)_3$	106	4	88.0	78–95
VIII	100	0.5% $Al_2(SO_4)_3$	106	4	88.2	76–102
IX	100	0.1% $AlCl_3$	106	4	88.0	81–97

Each animal was assigned an individual stanchion, which was given a number corresponding to the number on the animal. Each lot of animals had a separate loafing pen with free access to salt and clean, fresh water.

The concentrate mixture, consisting of 80 percent ground yellow corn and 20 percent wheat bran, was mixed every two weeks. Fluorine in the form of sodium fluoride was added in parts per million to the concentrate mixture, as shown in Table 32. The sodium fluoride added to each batch of feed was pre-mixed with a small amount of concentrate previous to mixing with the bulk of the concentrate. After mixing each batch of feed, the mixer was thoroughly cleaned to prevent any contamination. Aluminum sulfate ($Al_2(SO_4)_3 \cdot 18\ H_2O$) and aluminum chloride ($AlCl_3 \cdot 6\ H_2O$)[a] were added in the amounts as shown in Table 32, the percentage based on the total ration, and mixed with the concentrate as described for sodium fluoride. The concentrate mixture for each lot was then stored in separate galvanized containers.

Each animal was fed the concentrate mixture, and approximately one hour later the portion not consumed was removed from the feed box and weighed. After removing any concentrate which was refused, one-half pound of good quality chopped lespedeza hay was placed before each animal for approximately one hour, and then the hay weighback was taken. The animals were fed the same amount of concentrate and chopped lespedeza hay in the early morning and late evening from the beginning of the experiment until they were sacrificed. Every fourteen days the animals were individually weighed after the morning feeding, but before they had access to water.

Blood samples were taken periodically for the determination of calcium, phosphorus, potassium, sodium, and magnesium. After the blood samples were drawn, they were allowed to clot at room temperature, centrifuged, and serum removed. The Clark-Collip modification of the Kramer-Tisdall method, as described by Hawk *et al.* (1949), was used for the determination of serum calcium. Phosphorus was determined by a slight modification of the Fiske and Subbarow method as given by Hawk *et al.* (1949). Sodium and potassium in blood serum were determined by using the flame photometer according to the method described by Perkin-Elmar Instruction Manual (1949). The determination for serum magnesium was made according to a combination of methods described by Simorsen *et al.* (1947) and the modified Denis method as described by Hawk *et al.* (1949).

Every 28 days, two animals from each lot were placed on metabolism trials for seven days to obtain quantitative digestibility and mineral balance data. There was a three-day preliminary period before each metabolism collection to accustom the animals to the metabolism units. During the metabolism trials, the feces were weighed and the urine measured each morning.

Representative samples of concentrate, hay, feed weighback, urine, and feces were taken daily from each animal to make up individual composite samples which were analyzed for fluorine, calcium, phosphorus, nitrogen, crude fiber, ether extract, and nitrogen-free extract.

[a] Aluminum chloride hydrate will be referred to as aluminum chloride in the text.

Every 28 days, two animals selected at random from each of Lots I through VI and one animal each, except for the first period, from Lots VII through IX, were sacrificed. A gross autopsy was made on each animal. Bone, heart, liver, spleen, kidney, and lung samples were taken for pathological studies and fluorine analyses.

Because of hot weather, all animals that had not been sacrificed were sheared between the 84-day period and 112-day period.

During the third experimental period, rumen samples were taken, via stomach tube, from three animals from each of Lots I through VI and two animals each of Lots VII through IX. Gram stains were prepared of the original material and examined carefully for the morphological types of organisms present with special emphasis on the presence of the coccoid-type organism and the tiny gram positive curved rod. Also, the culturability and concentration of organisms were determined on samples from each animal.

Results and Discussion

Feed Consumption. At the time the experiment was initiated, the animals were consuming daily one pound of concentrate (80 percent ground yellow corn and 20 percent wheat bran) and approximately one pound of chopped lespedeza hay. The hay averaged 6 ppm fluorine (range 4 to 8 ppm F), and the grain for the control ration averaged 6 ppm fluorine (range 4 to 10 ppm F) throughout the experiment. Table 33 shows the average individual daily feed consumption for each lot per 28-day period throughout the experiment.

There was a slight decrease in the consumption of hay during the first 28-day period in the lots receiving the higher amounts of fluorine; however, Lot I (control) consumed slightly less than Lot II (25 ppm F added) or Lot III (50 ppm F added). Lot VI (200 ppm F added) and Lot VII (100 ppm F plus 0.1 percent aluminum sulfate) consumed less concentrate, especially during the first week, than the lots on lower levels of fluorine. Lot IX (100 ppm F plus 0.1 percent aluminum chloride) had a good feed consumption record in comparison with the other lots during this period.

Lot II (25 ppm F added) consumed slightly more hay and concentrate throughout the experiment than did Lot I (control) as shown in Table 33. Lot VI (200 ppm F added) consumed less hay and concentrate than the other lots. There was considerable variation in the amount of hay and concentrate consumed within each lot throughout the experiment, as noted throughout Table 33.

During the 140-day feeding trial with lambs, there was no significant decrease in feed consumption in Lots I through V (0 to 100 ppm F added); however, there was a significant decrease in Lot VI (200 ppm F added). In general, there was no significant decrease in feed consumption of animals

TABLE 33.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON FEED CONSUMPTION OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	Av. Daily Feed Consumed Per 28-Day Period—gms.									
			1		2		3		4		5	
			Hay	Grain	Hay	Grain	Hay	Grain	Hay	Grain	Hay	Grain
I	6		395	453	421	452	407	444	423	452	437	453
II	31		421	452	434	453	434	453	442	454	452	454
III	56		397	439	416	447	434	454	440	454	430	451
IV	81		379	426	408	449	408	444	420	453	397	434
V	106		379	431	408	452	418	453	435	454	445	452
VI	206		365	324	388	372	400	357	422	344	436	336
VII	106	0.1% $Al_2(SO_4)_3$	387	377	382	425	425	446	442	447	448	450
VIII	106	0.5% $Al_2(SO_4)_3$	402	414	406	422	431	438	440	448	430	447
IX	106	0.1% $AlCl_3$	425	445	421	444	442	452	434	454	441	454

in Lots VII, VIII, and IX (100 ppm F plus 0.1 percent and 0.5 percent aluminum sulfate for Lots VII and VIII, respectively, and 100 ppm F plus 0.1 percent aluminum chloride for Lot IX). This indicates that aluminum sulfate or aluminum chloride has little or no effect on feed consumption of lambs at this level for a period of 140 days.

Weights and Gains. The average beginning weight of the individual lots ranged from 88.0 to 88.4 pounds; however, there was considerable variation within each lot. Table 34 shows the average beginning weight, the average gain for the different periods, and the total average gain for the entire experiment.

During the first 28-day period, Lots III, IV and VII lost from 1.0 to 1.4 pounds and Lot VI lost 5.2 pounds, which corresponds very closely to feed consumption. All lots gained weight with the exception of Lot VI. In general, this corresponds to the increase in feed consumption during the second and third periods. All animals were sheared within the fourth period, which partially accounts for the large loss in weight of all lots. All lots gained during the fifth period; however, the alleviator groups gained more.

During the 140-day feeding trial, the lambs in Lots I and II (control and 25 ppm F added) gained slightly more than those in the other lots. The total average gains in Table 34 indicate there is considerable variation between lots and that 200 ppm fluorine added to the ration decreases the weight gains. Also, 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride fed to lambs receiving 100 ppm F for 140 days apparently did not materially affect weight gains.

Digestion and Balance Studies. A summary of the average percentage apparent digestibility for crude proteins, crude fiber, ether extract and nitrogen-free extract for sheep on various levels of fluorine is shown in Table 35. Column 4 in Table 35 gives the number of animals from each lot that were on 7-day metabolism trials. All hay, concentrate, weighback, feces and urine samples analyzed were a composite of 7-day collections.

There were some variations in the digestibility of the ration nutrients; however, these differences apparently were not associated with the fluorine content of the rations nor with the length of time the animals had been consuming the various rations. These digestibility results substantiate those previously reported on cattle. The addition of 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride to the ration of sheep receiving 100 ppm fluorine did not cause any appreciable differences in the digestibility of the various nutrients.

The fluorine, phosphorus, calcium and nitrogen balances for sheep on various levels of fluorine are shown in Tables 36 and 37, respectively, and for the convenience of presentation will be discussed separately. Column 4 of these tables gives the number of animals from each lot that were on 7-day metabolism trials. These data were determined from the samples collected

TABLE 34.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON WEIGHTS AND GAINS OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	Av. beginning wt. lbs.	Av. Gain Per 28-Day Period (lbs.)					Total Av. gain lbs.
				1	2	3	4[a]	5	
I	6		88.4	.8	7.0	5.0	−9.0	3.5	7.3
II	31		88.3	.6	6.6	4.0	−9.2	3.5	5.5
III	56		88.1	−1.4	7.9	2.8	−6.3	2.0	5.0
IV	81		88.2	−1.2	6.6	1.5	−9.8	2.5	−.4
V	106		88.3	0.0	6.1	2.5	−7.8	4.5	5.3
VI	206		88.3	−5.2	4.2	−.2	−7.4	1.0	−7.6
VII	106	0.1% $Al_2(SO_4)_3$	88.0	−1.0	7.8	2.9	−12.0	6.0	3.7
VIII	106	0.5% $Al_2(SO_4)_3$	88.2	1.0	4.2	4.0	−12.5	5.0	1.7
IX	106	0.1% $AlCl_3$	88.0	3.0	5.8	2.0	−13.0	7.0	4.8

[a] All animals were sheared between the 3rd and 4th periods, which accounts for some of the loss in weight and some of the variation.

during the metabolism trials, each collection being for a 7-day period. The feeds and feeding practices for animals on metabolism trials were the same as those for the remaining part of the experiment. Water was not considered in these balance studies because the same source of low fluorine water was used by animals in all lots.

TABLE 35.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON APPARENT DIGESTIBILITY OF NUTRIENTS OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. animals [a]	Percentage Digestibility: Crude protein	Crude fiber	Ether extract	Nitrogen free extract
0	— [b]		12	59.5	47.4	70.3	80.2
I	6		8	61.8	44.1	68.1	77.6
II	31		9	54.2	39.3	59.2	75.9
III	56		8	61.2	50.6	72.0	79.1
IV	81		8	59.6	49.4	70.6	78.5
V	106		7	61.9	51.2	68.0	80.4
VI	206		8	56.6	49.3	70.4	78.0
VII	106	0.1% $Al_2(SO_4)_3$	7	56.6	47.4	69.0	75.6
VIII	106	0.5% $Al_2(SO_4)_3$	7	59.6	49.0	69.4	76.7
IX	106	0.1% $AlCl_3$	7	58.6	46.2	64.0	70.4

[a] Each animal was on trial for 7 days.
[b] Pre-experimental.

Fluorine balance. The average daily milligrams of ingested, urinary, fecal and retained fluorine for animals on various levels of fluorine are shown in Table 36. Generally, the milligrams of ingested fluorine increase the amount of fluorine added to the ration.

Collections made during the first and second periods indicated the fluorine intake of animals from Lots I through V (0 to 100 ppm F added) corresponded with the amount added to the ration, but the fluorine intake of animals from Lot VI (200 ppm F added) was less than that of animals from Lot V (100 ppm F added). This can be explained partially by the lower feed consumption of animals from Lot VI during the first and second experimental periods. Ingested fluorine among animals of Lots I through VI increased with the fluorine content of rations for the third, fourth, and fifth periods, with the exception of Lots III and IV during the fourth period and Lot IV during the fifth period. The fluorine intake record of Lots VII, VIII, and IX corresponds very closely with that of Lot V, indicating that 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride has very little effect on feed consumption of animals receiving 100 ppm fluorine added to their rations.

Table 36.—Effects of Fluorine and Fluorine Plus Alleviators on Fluorine and Phosphorus Balances of Lambs in Experiment X, Lots 1–9

Lot no.	Total F in ration ppm	Alleviator percent	Fluorine—mgs. No. animals [a]	Fluorine—mgs. Intake	Fluorine—mgs. Urine	Fluorine—mgs. Feces	Fluorine—mgs. Retained	Fluorine Retained percent	Phosphorus—gms. No. animals [a]	Phosphorus—gms. Intake	Phosphorus—gms. Urine	Phosphorus—gms. Feces	Phosphorus—gms. Retained	Phosphorus Retained percent
0	— [b]		12	3.6	.6	1.4	1.6	44.4	12	3.24	.01	2.55	.68	21.0
I	6		10	6.1	3.8	2.3	0.0	0.0	8	3.74	.01	2.70	1.03	27.5
II	31		10	27.4	9.1	3.6	14.7	53.6	8	3.78	.01	2.74	1.03	27.2
III	56		9	40.6	10.4	5.9	24.3	59.8	7	3.74	.01	2.39	1.34	35.8
IV	81		10	74.9	11.8	5.9	57.2	76.4	8	3.58	.02	2.18	1.38	38.5
V	106		9	86.1	22.1	7.6	56.4	65.5	7	3.80	.03	2.18	1.59	41.8
VI	206		10	107.4	18.6	11.3	77.5	72.2	8	2.92	.03	1.76	1.13	38.7
VII	106	0.1% $Al_2(SO_4)_3$	7	95.0	10.9	20.7	63.4	66.7	7	3.13	.01	2.73	.39	12.5
VIII	106	0.5% $Al_2(SO_4)_3$	7	101.8	11.3	27.5	63.0	61.9	7	3.11	.01	2.59	.51	16.4
IX	106	0.1% $AlCl_3$	7	92.5	11.3	33.3	47.9	51.8	7	3.12	.02	2.52	.58	18.6

[a] Each animal was on trial for 7 days.
[b] Pre-experimental.

The average amount of urinary fluorine increased as the fluorine content of the rations increased for Lots I through V (0 to 100 ppm F added); however, some of the individual analyses were not consistent. This is in concordance with work on cattle reported by Hobbs *et al.* (1951), indicating that urinary fluorine may assist in determining the level of fluorine being consumed at a given time, provided the level is low and a large number of animals are sampled. Urinary fluorine of animals from Lot VI (200 ppm F added) was less than that of animals from Lot V (100 ppm F added). This indicates that total urinary fluorine of animals on high fluorine intake (200 ppm F added) may not be a very reliable estimate of fluorine consumption; however, it is granted that only ten seven-day collections provide limited data.

Urine collections were taken from animals in Lots VII, VIII and IX during all periods except the first, and the analyses showed that urinary fluorine was lower for each period than that from animals in Lot V. Table 36 shows that animals from Lots VII, VIII and IX excreted approximately one-half as much urinary fluorine as did animals from Lot V. This indicates that 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride significantly decreases the urinary fluorine of animals receiving 100 ppm fluorine added to the ration.

The total fecal fluorine of animals from Lots I through VI generally increased as the fluorine content of the ration increased (Table 36); however, there was considerable variation by different animals and for different periods. This is in partial concordance with theories that total fecal fluorine may be used as an aid in determining levels of currently ingested fluorine, provided large numbers of animals are sampled.

Average daily fecal fluorine of animals from Lots VII, VIII and IX was approximately three to four times that of animals from Lot V (Table 36). This indicates that 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride ties up ingested fluorine in the gastro-intestinal tract with the aluminum chloride showing some advantage over the aluminum sulfate. This is in concordance with the decreased urinary fluorine of animals from these lots.

In general, the total retained fluorine increased as the fluorine content of the ration increased, although there was a great amount of variation between animals and periods of time. Part of this variation may have occurred because the retained fluorine was determined by subtracting the urinary and fecal fluorine from the total ingested fluorine, thereby increasing the possibility of determination error. The addition of 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride to the ration of sheep receiving 100 ppm fluorine did not indicate any significant effect upon fluorine retention. This is not in concordance with the 20 to 30 percent reduction in the fluorine content of bones from animals receiving the aluminum compounds as compared to those receiving the same amount of fluorine with no alleviators (Table 39). However, it should be kept in mind that a balance trial is over a very short period of time and, at best, under some abnormal conditions. Mineral studies with sheep, cattle and swine should be conducted over a good part of an animal's productive life for most useful results to the livestock industry.

Phosphorus balance. The average daily grams of ingested, urinary, fecal and retained phosphorus are shown in Table 36. The phosphorus content of the samples collected from animals of Lot I through Lot VI during the third metabolism trial is not included in this table because of an unavoidable accident after the samples were collected.

The ingested phosphorus of animals from Lot VI (200 ppm F added) was lower than that of other lots, mainly because of lower feed consumption. Table 36 shows that the animals from Lots VII, VIII and IX had slightly more phosphorus in the feces, less retained, and a lower percent retained than that of animals from Lot V. This may be explained partially by the lower phosphorus intake; and/or it may have been caused by the addition of aluminum sulfate or aluminum chloride to the ration.

Calcium balance. Table 37 gives the average daily grams of ingested, urinary, fecal and retained calcium for sheep fed various levels of fluorine and alleviators. There were no significant differences in the average daily ingested, urinary, fecal or retained calcium that could be associated with the level of fluorine intake, except that the ingested and retained calcium for Lot VI was lower than that of the other lots. This can be explained partially by the lower feed consumption of animals in Lot VI. The calcium balance did not appear to be affected by the use of dietary aluminum sulfate or aluminum chloride.

Nitrogen balance. The differences in nitrogen intake, excretion and retention (Table 37) were apparently not associated with the fluorine content of rations consumed, except that animals in Lot VI had a lower nitrogen intake, excretion and retention than those on lower levels of fluorine. This might be the result of a lower feed consumption.

The fecal nitrogen was slightly greater and the urinary and retained nitrogen slightly less for animals in Lot V (100 ppm F added) than that of animals in Lots VII, VIII and IX (100 ppm F added plus 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride, respectively). However, this is a small difference and is not in concordance with the digestibility of crude protein.

Blood Studies. Blood samples were taken from all animals in each lot at various intervals throughout the experiment. The samples were allowed to clot, then they were centrifuged, the serum was removed, and samples were analyzed for phosphorus, sodium, potassium, magnesium and calcium. Table 38 shows the average analyses of the entire experiment for each lot.

TABLE 37.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON CALCIUM AND NITROGEN BALANCES OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. animals [a]	Calcium—gms. Intake	Calcium—gms. Urine	Calcium—gms. Feces	Calcium—gms. Retained	Retained percent	No. animals [a]	Nitrogen—gms. Intake	Nitrogen—gms. Feces	Nitrogen—gms. Urine	Nitrogen—gms. Retained	Retained percent
0	— [b]		12	4.45	.08	3.74	.63	14.2	12	15.23	6.16	4.33	4.74	31.1
I	6		10	4.51	.08	3.28	1.15	25.5	10	14.50	5.57	3.96	4.97	34.3
II	31		10	4.41	.06	3.34	1.01	22.9	10	14.50	6.44	4.53	3.53	24.3
III	56		9	4.50	.06	3.12	1.32	29.3	9	14.54	5.67	4.65	4.22	29.0
IV	81		10	4.29	.03	2.92	1.34	31.2	10	13.94	5.64	4.11	4.19	30.1
V	106		9	4.35	.02	3.04	1.29	29.7	9	14.28	5.63	4.82	3.83	26.8
VI	206		10	3.67	.02	2.99	.66	18.0	10	11.78	5.08	3.72	2.98	25.3
VII	106	0.1% $Al_2(SO_4)_3$	7	4.85	.05	3.18	1.62	33.4	7	14.65	4.81	5.38	4.46	30.4
VIII	106	0.5% $Al_2(SO_4)_3$	7	4.86	.09	3.28	1.49	30.7	7	14.27	4.90	5.08	4.29	30.1
IX	106	0.1% $AlCl_3$	7	4.79	.04	3.13	1.62	33.8	7	15.46	4.68	5.73	5.05	32.7

[a] Each animal was on trial for 7 days.
[b] Pre-experimental.

TABLE 38.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON BLOOD FROM LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. samples taken	Phosphorus mg. P/100 ml. serum	Sodium mg. Na/100 ml. serum	Potassium mg. K/100 ml. serum	Magnesium mg. Mg/100 ml. serum	Calcium mg. Ca/100 ml. serum
I	6		52	7.42	344.8	24.11	2.10	12.19
II	31		52	7.85	339.6	23.44	2.07	11.71
III	56		48	7.69	342.5	23.43	2.05	11.68
IV	81		52	8.35	339.4	22.47	2.12	11.81
V	106		52	9.21	343.3	24.89	2.36	11.48
VI	206		52	8.98	342.2	24.07	2.09	11.60
VII	106	0.1% $Al_2(SO_4)_3$	24	8.20	343.4	28.01	2.14	12.10
VIII	106	0.5% $Al_2(SO_4)_3$	24	7.89	340.6	24.36	2.18	12.71
IX	106	0.1% $AlCl_3$	24	8.37	341.1	23.54	2.20	12.13

Column 4 in Table 38 gives the total number of samples analyzed from each lot. It is noted that all analyses fall within the normal range for sheep at this age according to Dukes (1947).

Differences are noted in these analyses; however, these differences cannot be associated with the fluorine content of the rations. It is noted in Table 38 that blood serum from animals receiving the higher levels of fluorine had a slightly higher phosphorus content than that of animals on a control ration.

These analyses indicated that the fluorine content of feed, up to 200 ppm fluorine, did not affect the phosphorus, sodium, potassium, magnesium, or calcium level of blood serum from lambs on these treatments over a period of 140 days. Also, 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, or 0.1 percent aluminum chloride, in the ration, apparently did not show any consistent differences in animals receiving 100 ppm fluorine for a period of 140 days.

Fluorine Content of Bones. Table 39 shows the fluorine content of a sagittal section of the metacarpals and the angle of the mandible bones from lambs sacrificed at the end of each experimental period. These results are reported in ppm fluorine on an ash basis. Column 3 in Table 39 gives the number of animals sacrificed at the end of each period except as noted. No animals were killed from Lots VII, VIII and IX at the end of the first period, because only four animals were used in each of these lots, and it was assumed more valuable results could be obtained from the alleviator groups by holding them until the later periods.

The fluorine content of the mandibles was higher than the fluorine content of metacarpals of all animals, which is in concordance with other work on storage of fluorine in bones of animals that have been receiving rations containing large amounts of fluorine. Isotope studies with various minerals have substantiated these bone differences, and effects of time, on a given ration.

The base fluorine content of the bones increased as a function of intake and time on a given ration at each of the five experimental periods. Also, the fluorine content of bones increased with the length of time on a given ration, with the exception of Lot IV during the third period, and this value was exceptionally high because the fluorine concentration in bones from one animal was very high.

The mandibles and metacarpals of animals for Lots VII, VIII and IX (100 ppm F added plus alleviators) had a lower fluorine concentration for all periods than those of animals in Lot V (100 ppm F added). At the end of the second period this decrease in fluorine concentration was small for the alleviator groups; however, at the end of the fifth period it was approximately 15 percent for animals receiving 0.1 percent aluminum sulfate, approximately 35 percent for the animals receiving 0.5 percent aluminum

TABLE 39.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON BONE FLUORINE CONTENT OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. animals killed each period	Fluorine Content of Bones [a] Per 28-Day Period: 1		2		3		4		5	
				Meta-carpal	Man-dible	Meta-carpal	Man-dible	Meta-carpal	Man-dible	Meta-carpal	Man-dible	Meta-carpal	Man-dible
I	6 [b]		2	285	345	440	530	465	745	605	915	675	940
II	31		2	835	1025	1100	1450	1150	1800	1500	2000	1500	2350
III	56		2	895	1350	1633 [c]	2133 [c]	2200	2800	1800	3350	1900 [d]	4000 [d]
IV	81		2	1210	1550	2750	3250	3300	4500	3200	4600	3800	5850
V	106		2	1050	2200	3000	3900	3450	5300	3500 [d]	6350	4950	8050
VI	206		2	1650	3400	3150	5550	3100	6850	4900	8300	6500	9500
VII	106	0.1% $Al_2(SO_4)_3$	1	—	—	2600	3700	3600	5000	3400	5800	4200	6400
VIII	106	0.5% $Al_2(SO_4)_3$	1	—	—	2700	3400	2400	3700	3000	4900	3200	5200
IX	106	0.1% $AlCl_3$	1	—	—	2800	3600	2800	4000	3000	4800	3400	5900

[a] Reported in ppm on ash basis.
[b] Pre-experimental lambs' legs averaged 470 ppm F and jaw averaged 497 ppm F.
[c] Average of three animals, but one animal was killed 45 days after experiment started.
[d] One animal.

sulfate, and approximately 30 percent for the animals receiving 0.1 percent aluminum chloride. This shows that storage of fluorine in bone can be lowered by the addition of either aluminum sulfate or aluminum chloride to the ration of lambs ingesting increased levels of fluorine.

Histological sections of the jaw and leg bones did not show any significant differences that could be associated with the various treatments.

Fluorine Content of Soft Tissues. The average fluorine content of livers, hearts, kidneys, lungs, and spleens of animals sacrificed during the pre-experimental period and at the end of the first and fourth periods is shown in Table 40 in ppm fluorine on a wet basis. Fluorine determinations were made on tissues from one animal each of Lots VII, VIII and IX. These animals were sacrificed at the end of the fourth period, consequently providing a limited amount of data on these lots.

Differences were noted in the fluorine content of the various tissues; however, these differences apparently were not associated with fluorine intake. The kidneys from animals in Lot VI, sacrificed at the end of the fourth period, contained a higher level of fluorine than those of animals in other lots, which may be due to urine in the kidney that was high in fluorine. The livers, lungs, and spleens from animals of Lots VII, VIII and IX that were sacrificed at the end of the fourth period were lower in fluorine concentration than the tissues of corresponding animals of Lot V; however, these data are too limited to prove that the difference was due to the alleviators.

Histological sections were made of livers, hearts, kidneys, lungs, and spleens from lambs on the various treatments. These sections did not show significant differences that could be associated with the various treatments under the conditions of this experiment.

Urinary Fluorine. Many workers have considered urinary excretion of fluorine by an animal as an indication of the amount of fluorine ingested. Blakemore *et al.* (1948) reported that the urinary level at any given time is influenced not only by the current rate of absorption from food, but also by the rate of excretion from the skeleton of fluorine stored in the past. Also, it is very important to consider the fluorine content of the present and past rations, length of time animals have been on a particular ration, age of the animal, and age of the animal when put on the ration in question.

Table 41 shows the average ppm fluorine in urine, overall average fluorine content, and the average daily milligrams of fluorine in urine from lambs fed known levels of fluorine and alleviators for different intervals of time. Considerable variation is noted in the fluorine content of urine from one period to another. In general, the fluorine content of urine during the last period is higher than that of the other periods.

In Figure 1 the ppm fluorine in the ration and the ppm fluorine in urine are plotted in broken lines and the milligrams of fluorine consumed and

TABLE 40.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON TISSUE FLUORINE CONTENT OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. animals	Fluorine Content of Tissues Per 28-Day Period (ppm) [a]									
				1					4				
				Liver	Heart	Kidney	Lung	Spleen	Liver	Heart	Kidney	Lung	Spleen
I	6 [b]		2	.23	.36	1.04	.54	.43	.30	.34	1.32	.59	.62
II	31		2	.63	.92	1.11 [b]	.48	.71	1.64	.54	1.16	.48	.44
III	56		2	.33 [b]	.14 [b]	1.27 [b]	.40 [b]	.65 [b]	.21	.49	1.22 [b]	1.53 [b]	.76
IV	81		2	.34	.21	.86 [b]	.28	.58	.34	.40	1.68	.38	.53
V	106		2	.16	.11	.43	.27	.05	.62	.66	1.29	.80	1.08
VI	206		2	.47	.47 [b]	1.42	.05	.50	.42	.48	2.73	.81	.44
VII	106	0.1% $Al_2(SO_4)_3$	1	—	—	—	—	—	.55	.92	1.57	.75	.54
VIII	106	0.5% $Al_2(SO_4)_3$	1	—	—	—	—	—	.31	.97	1.20	.56	.50
IX	106	0.1% $AlCl_3$	1	—	—	—	—	—	.50	.42	.85	.38	.49

[a] Analysis reported on basis as taken.

[b] Two pre-experimental animals' tissues averaged as follows: Liver, 0.89 ppm F; heart, 1.5 ppm F; kidney, 3.1 ppm F; lung, 0.92 ppm F; spleen, 0.92 ppm F.

TABLE 41.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON URINE FLUORINE CONTENT OF LAMBS IN EXPERIMENT X, LOTS 1–9

Lot no.	Total F in ration ppm	Alleviator percent	No. animals [a]	F content periodically—ppm 21 Days	54 Days	83 Days	112 Days	143 Days	Av. F content in urine ppm	Av. F content in urine per day mgs.
I	6 [b]		2	3.0	11.0	7.0	3.5	7.5	6.4	3.8
II	31		2	36.5	22.5	17.0	11.5	34.0	24.3	9.1
III	56		2	43.0	34.0	35.0	10.0	49.0	32.6	10.4
IV	81		2	68.5	58.0	58.0	24.5	60.0	53.8	11.8
V	106		2	94.5	77.5	62.0	39.5	41.0 [g]	65.3	22.1
VI	206		2	91.5	53.0	48.5	37.0	72.0	60.4	18.6
VII	106	0.1% $Al_2(SO_4)_3$	2	—	65.5 [c]	6.7 [d]	33.5 [e]	50.0 [f,g]	37.3	10.9
VIII	106	0.5% $Al_2(SO_4)_3$	2	—	66.5 [c]	11.7 [d]	37.0 [e]	65.0 [f,g]	42.2	11.3
IX	106	0.1% $AlCl_3$	2	—	56.5 [c]	10.5 [d]	29.5 [e]	54.0 [f,g]	35.3	11.3

[a] Each animal was on trial for 7 days during each period.
[b] Urine from twelve pre-experimental animals averaged 4.4 ppm F.
[c] 41 days from beginning of experiment.
[d] 69 days from beginning of experiment.
[e] 103 days from beginning of experiment.
[f] 133 days from beginning of experiment.
[g] One animal.

CORRELATION OF FLUORINE IN RATIONS TO URINARY FLUORINE IN LAMBS

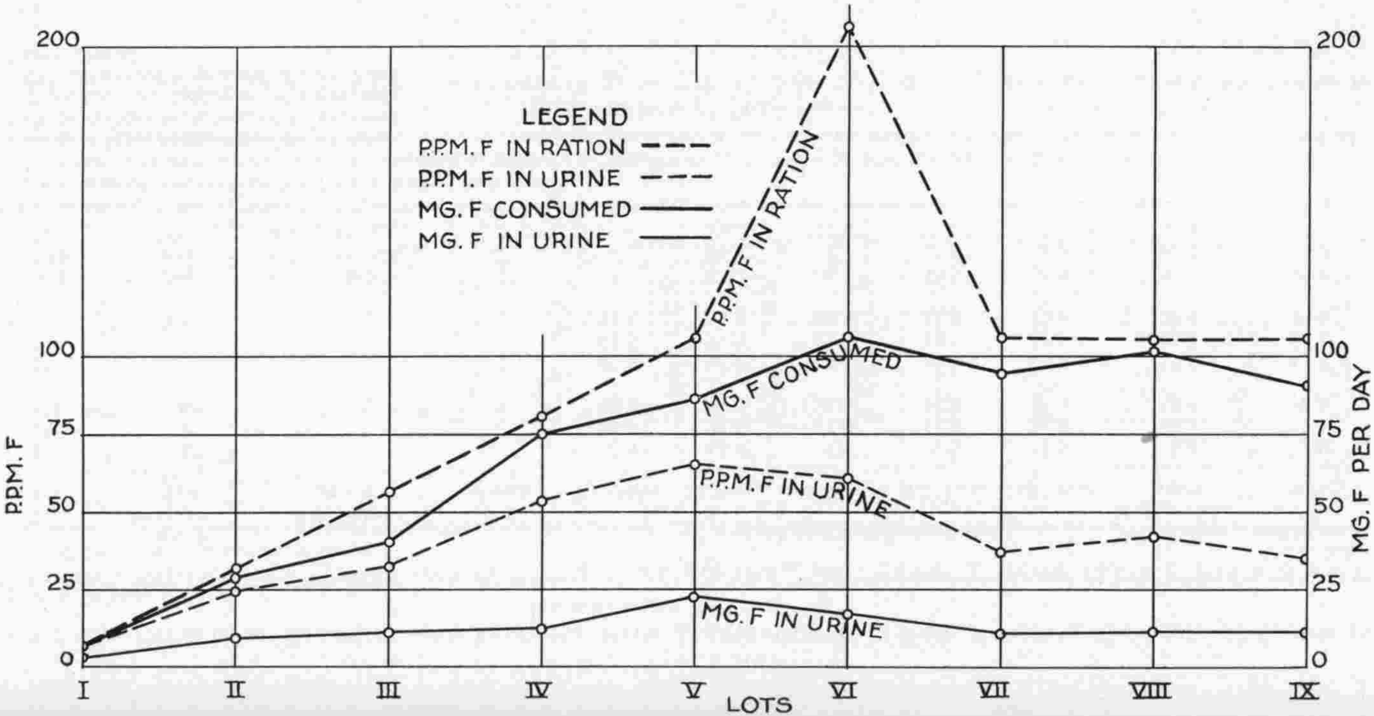


FIGURE I.

milligrams of urinary fluorine are plotted in solid lines for the entire experiment. The correlations of the plotted factors are as follows:

	Lots I–IX	Lots I–VI
ppm F in urine vs. ppm F in current ration	.778 [a]	.816 [b]
ppm F in urine vs. mgs. F in urine	.927 [a]	.933 [b]
ppm F in urine vs. mgs. F consumed	.778 [a]	.965 [b]
mg. F in urine vs. ppm F in current ration	.741 [a]	.798 [b]

[a] significant at .01 level
[b] significant at .05 level

It was necessary to determine the correlations of these factors for Lots I through VI and also for Lots I through IX because alleviators were used in the rations of animals in Lots VII through IX. In using these correlations, it is very important to keep in mind the alleviators used and also the fluorine content of bones as given in Table 39.

This statistical analysis showed that the fluorine content of rations and urinary fluorine are correlated significantly at either the .01 or .05 level. However, it should be emphasized that the fluorine content of urine alone should not be used to determine the approximate amount of fluorine consumed at a given time. The urinary fluorine of an animal also may be influenced by the rate of excretion from the skeleton of fluorine stored in the past, amount and rate of fluorine in rations previously consumed, length of time on a given ration, age of the animal, and age of the animal when put on a ration containing increased amounts of fluorine.

Rumen Bacteriological Studies. Bacteriological studies were conducted on three animals each from Lots I through VI and two animals each from Lots VII through IX. The rumen samples were taken approximately 60 days after the animals were put on various levels of fluorine and alleviators. Gram stains were examined in the usual manner and no significant decrease appeared in the concentration of the coccoid-type organism or in the tiny gram positive curved rods. The degree of parasitism of fibers appeared to be uniformly heavy for all lots.

Bacteriological slide counts, which averaged about 60 billion per gram, were uniformly higher for the control animals than for those receiving fluorine. Animals receiving up to 200 ppm fluorine maintained a count of approximately 35 to 40 billion per gram, but animals receiving 200 ppm fluorine showed about 25 billion per gram. Animals receiving 100 ppm fluorine plus alleviators averaged about 30 billion per gram. Since the animals had been receiving the fluorine for only a short period of time, this might be only a temporary effect and the rumen flora may adjust itself to the conditions imposed by the presence of fluorine.

There was no apparent difference between lots in the height of cultural growth or the distribution of the various types of organisms isolated. Animals receiving 100 ppm fluorine with and without aluminum compounds gave a similar bacteriological picture in respect to their rumen contents.

Summary

The effects of various levels of fluorine and of alleviators upon feeder lambs were studied periodically over a period of 140 days. The basal ration consisted of one pound of concentrate (80 percent ground yellow corn and 20 percent wheat bran) and one pound of chopped lespedeza hay per animal per day.

Sodium fluoride was added to the concentrate mixture of the first six lots (I through VI) in the following amounts: 0, 25, 50, 75, 100, and 200 ppm F, respectively. Lots VII through IX received 100 ppm F added as sodium fluoride plus 0.1 percent aluminum sulfate, 0.5 percent aluminum sulfate, and 0.1 percent aluminum chloride, respectively. The basal hay and concentrate averaged 6 ppm fluorine.

Under the conditions of the experiment, the lambs receiving 200 ppm fluorine consumed slightly less feed and gained slightly less weight than animals in the other lots. The apparent digestibility values of crude protein, crude fiber, ether extract, and nitrogen-free extract did not show significant differences that could be attributed to the various treatments.

In general, as the level of fluorine intake increased, the retained fluorine increased, as measured by bone storage and by balance studies. Calcium and nitrogen balance studies did not show significant differences that could be attributed to the intake of fluorine or alleviators used in this study. Differences were noted in the phosphorus balance studies; however, these differences apparently were not directly associated with the fluorine intake, but there were indications that the alleviators used in this study might have increased the phosphorus excretion.

Sodium, potassium, magnesium and calcium levels in blood from these lambs showed no apparent differences that could be associated with the various levels of fluorine and alleviators.

The concentration of fluorine in bones of all lambs increased as a function of time and the level of fluorine intake. Bones from lambs receiving 100 ppm fluorine plus alleviators had a lower fluorine concentration than those of lambs fed similar rations without alleviators.

Levels of fluorine and alleviators fed to lambs were not measurably reflected by the fluorine concentration in soft tissues, including the liver, heart, kidney, lungs, and spleen.

In general, the urinary fluorine increased as the fluorine intake increased,

and the alleviators used in this study decreased the urinary fluorine as compared to urine from lambs receiving a similar treatment without alleviators.

Pathological and rumen bacteriological studies did not show consistent differences that could be correlated with levels of fluorine intake nor alleviators; however, there may have been some indication of a decrease in the concentration of rumen micro-organisms in lambs receiving the higher levels of fluorine.

EXPERIMENT XI—LOTS 1–6

Objectives

The purpose of this experiment was to study the several physiological changes associated with the feeding of various levels of fluorine (0–100 ppm F), as sodium fluoride, to breeding ewes and the effectiveness of aluminum sulfate as an alleviator of the effects of fluorine.

The several phases of the experiment included effects on:

1. Feed consumption.
2. Growth and gains.
3. Reproduction of ewes.
4. Fluorine concentration of the bones of ewes and their lambs.

Experimental Procedure

Thirty-six western crossbred ewe lambs, eight to twelve months of age and averaging approximately 80 pounds in weight, were used in this study. They were ewe lambs from the same source as the wether lambs used in Experiment X. The lambs were carried through a pre-experimental period in an effort to rid them of internal parasites and get them on feed. During this period the daily ration of chopped hay and ground yellow corn was increased until the animals were consuming approximately one and one-half pounds of hay and one-half pound of concentrate per animal per day at the time the experiment began.

These lambs were allotted on the basis of weight, grade and condition into six lots with six animals per lot. Each animal was identified with a numbered ear tag and a paint brand.

Each group was placed in a dry lot with free access to water and salt. The rations consisted of chopped hay containing at least 50 percent alfalfa fed *ad lib.*, and one-half pound of No. 2 ground yellow corn. The levels of fluorine and aluminum sulfate added are shown in Table 42. The methods of calculating these additives and mixing the concentrates were the same as described for cattle. The ewes were group fed the grain and chopped hay once a day. Refused portions were collected, weighed, and recorded by lots daily.

TABLE 42.—PLAN OF EXPERIMENT XI—SHEEP, LOTS 1–6

Lot no.	F added in ration ppm	Alleviator percent	Total F in ration ppm	No. animals	Av. initial wt. lbs.	Av. daily Mg. F/Kg. body wt.
I	0		11	6	79.2	.25
II	25		36	6	81.2	.91
III	50		61	6	82.2	1.65
IV	50	0.2% $Al_2(SO_4)_3$	61	6	81.3	1.62
V	100		111	6	81.0	3.00
VI	100	0.4% $Al_2(SO_4)_3$	111	5 [a]	85.4 [b]	2.89

[a] One animal in this lot was sacrificed 29 months after experiment began.
[b] One animal died 2 months after experiment began. The data for this animal was deleted from the experiment.

These animals were weighed, their teeth examined, and color photographs made of the incisors.

This experiment was started at the Middle Tennessee Experiment Station at Columbia, Tennessee, in December, 1949. In June, 1951, the ewes were brought to the Knoxville Station where they were continued on test until the experiment was terminated in December, 1952. These ewes lambed in 1951 and in 1952. At the termination of the experiment, these animals were slaughtered in the University meats laboratory. All ewes were autopsied and bone samples were taken from the right metacarpal, mandible, and right ninth rib. Fluorine determinations were made on these samples.

Results and Discussion

Feed Consumption. At the time this experiment was initiated, the animals were consuming approximately one and one-half pounds of hay and one-half pound of concentrate daily. During 1952 the average daily feed consumption, as shown in Table 43, increased from one and one-half pounds to approximately three pounds of hay. Since the lambs were in the lots with the ewes, they consumed some of the same feed as the ewes in the respective lots. During the first two years, there was practically no difference in hay or concentrate consumption between any of the lots. In 1952, however, there was a reduction in the hay consumption in Lot VI.

The average fluorine content of the hay fed these animals was 9.9 ppm (range 4 to 38 ppm F) and the concentrate for the control group averaged 17 ppm (only three analyses with a range of 8 to 31 ppm F). The average daily consumption of fluorine in milligrams per kilogram of body weight and consumption of aluminum sulfate by these animals is shown in Table 42. The level of fluorine fed had no apparent effect upon feed consumption. At the 50 ppm level (Lots III and IV) the addition of .2 percent aluminum sulfate had no effect on feed consumption when compared to the ration void of this compound.

TABLE 43.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON FEED CONSUMPTION OF EWES IN EXPERIMENT XI, LOTS 1–6

Lot no.	Total F in ration ppm	Alleviator percent	1950		1951		1952		1950 through 1952	
			Hay	Conc.	Hay	Conc.	Hay	Conc.	Hay	Conc.
I	11		1.98	.49	2.98	.50	2.98	.50	2.66	.50
II	36		1.94	.49	2.98	.50	2.97	.50	2.64	.50
III	61		1.93	.49	2.98	.50	2.97	.50	2.64	.50
IV	61	0.2% $Al_2(SO_4)_3$	1.96	.48	2.98	.50	2.97	.50	2.65	.50
V	111		1.95	.48	2.98	.50	2.91	.50	2.63	.50
VI [a]	111	0.4% $Al_2(SO_4)_3$	2.06	.52	2.99	.50	2.52	.47	2.52	.50

[a] Only 4 animals.

Weights and Gains. The average daily gains of the sheep are reported in Table 44. Table 42 shows the average initial weight and range by lots.

TABLE 44.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON WEIGHTS AND GAINS OF EWES IN EXPERIMENT XI, LOTS 1-6

Lot no.	Total F in ration ppm	Alleviator percent	Av. daily gains			
			1950 344 days	*1951* 352 days	*1952* 387 days	*1950–1952* 1083 days
I	11		.10	.04	−.01	.04
II	36		.10	.07	−.06	.04
III	61		.10	.04	−.04	.03
IV	61	0.2% $Al_2(SO_4)_3$	.11	.02	.00	.04
V	111		.11	.04	−.06	.03
VI [a]	111	0.4% $Al_2(SO_4)_3$	.14	.00	.00	.04

[a] Only 4 animals.

All lots show weight losses for the year of 1952. This might be partially attributed to the fact that these ewes were nursing lambs. Griffith (1953) also substantiates this for a 140-day period by reporting no material effect on weight gains with wether lambs fed similar rations.

Reproduction and Lamb Records. The ewes used in this experiment lambed in 1951 and 1952. The ewes were at the Middle Tennessee Experiment Station at Columbia, Tennessee, when they lambed in 1951. There were no significant differences between lots in the birth weights of the lambs produced in both years and no differences in average daily gain in the 1952 lambs. The level of fluorine apparently did not reduce the number of lambs born since Lot V had 92 percent lamb crop and Lot VI had 88 percent lamb crop for the two years. The average lamb crop for all lots for the two years was 68 percent. The summary of the birth weights for 1951 and 1952 and the average daily gains of these lambs is shown in Table 45.

Fluorine Content of Bones. Results of the average analysis of samples from the metacarpals, mandibles and ninth ribs of these sheep are shown in Table 46. The metacarpal and mandible samples were taken in the same manner as for the calves. The rib sample was obtained from the distal half of the right ninth rib.

The addition of 0.2 percent aluminum sulfate significantly reduced the fluorine storage in the ribs of these sheep at the 50 ppm fluorine level (Lot III compared to Lot IV). Reductions were also obtained when 0.4 percent aluminum sulfate was added to the ration of sheep fed fluorine at the 100 ppm level (Lot V compared to Lot VI) as shown in Table 46. The average rib analysis of Lot VI (100 ppm F + 0.4 percent aluminum sulfate added) was almost as low as the average rib analysis of Lot III (50 ppm F added).

Fluorine storage in the mandibles was significantly reduced at the 50 ppm fluorine added level by the addition of 0.2 percent aluminum sulfate

TABLE 45.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALUMINUM SULFATE ON REPRODUCTION AND LAMBS OF EWES IN EXPERIMENT XI, LOTS 1–6

Lot no.	Total F in ration ppm	Alleviator percent	1951		1952			Total no. born	Total no. raised
			No. born	Av. birth wt.	No. born	Av. birth wt.	Av. daily gain [a]		
I	11		5	7.2	2	7.8	.31	7	5
II	36		2	8.2	4	9.4	.44	6	5
III	61		4	7.3	4	10.6	.36	8	7
IV	61	0.2% $Al_2(SO_4)_3$	5	9.2	2	10.4	.33	7	5
V	111		5	6.7	6	8.2	.38	11	7
VI [b]	111	0.4% $Al_2(SO_4)_3$	5	7.8	3	8.2	.32	8	5

[a] Average daily gains available only on 1952 lambs.
[b] Only 4 animals.

Table 46.—Effects of Fluorine and Fluorine Plus Aluminum Sulfate on Bone Fluorine Content of Lambs and Ewes in Experiment XI, Lots 1–6

Lot no.	Total F in ration ppm	Alleviator percent	Ewe data: F content in metacarpal	Ewe data: F reduction percent	Ewe data: F content in mandible	Ewe data: F reduction percent	Ewe data: F content in rib	Ewe data: F reduction percent	Lamb data: No. of lambs	Lamb data: Av. F content in metacarpal	Lamb data: Range
I	11		937		1317		1300 [a]		2	172	130–213
II	36		2733		5233		5400 [b]		4	299	236–385
III	61		4367		8733		9480 [b]		4	578	520–640
				9.6		13.3		30.9			
IV	61	0.2% $Al_2(SO_4)_3$	3950		7567		6550 [c]		2	598	480–715
V	111		7917		12000		12740 [d]		6	1065	850–1440
				44.7		22.7		23.9			
VI	111	0.4% $Al_2(SO_4)_3$	4375 [c]		9275		9725 [c]		3	1026	945–1035

[a] One animal of 8300 ppm F was not used in this average.
[b] Six analyses.
[c] Only 4 analyses.
[d] Only 5 analyses.

when compared to the ration that contained no aluminum sulfate (Lot III). It also should be noted that the addition of 0.4 percent aluminum sulfate to a ration containing 100 ppm fluorine added (Lot VI) produced a high percentage decrease in fluorine content of the mandibles when compared to a ration containing the same amount of fluorine but no aluminum sulfate (Lot V). Reductions in the fluorine content of the metacarpals were observed with such wide variations that statistical analysis of the data did not show a significant difference. The average of analyses of the metacarpals of Lot VI (100 ppm F + 0.4 percent aluminum sulfate added) is practically the same as that of Lot III (50 ppm F added).

These data would indicate that under the conditions of this experiment 0.2 percent aluminum sulfate significantly reduced the fluorine storage in the right ninth rib and mandible of ewes receiving a ration with 50 ppm F added over a three-year period. Also, high percentage reductions in fluorine storage in the right ninth rib, mandible and metacarpal were obtained by the addition of 0.4 percent aluminum sulfate to a ration that had 100 ppm fluorine added.

The results of analyses of the right metacarpals of the lambs produced from the ewes in Lots I through VI in 1952 are shown in Table 46. Since these lambs were kept in the same lots as the ewes, they consumed some of the feed. Thus, the increases in the amount of fluorine in the metacarpals of these lambs parallel the increases in the rate of fluorine added to the rations of the various lots.

Summary

The effects of fluorine and the possible alleviation of these effects with aluminum sulfate were studied in 34 ewes. The fluorine was added at the rate of 0, 25, 50, and 100 ppm with 0.2 percent aluminum sulfate being added to a ration with 50 ppm F, and 0.4 percent aluminum sulfate added to a ration containing 100 ppm F.

These data show that, under the conditions of this experiment, there were no differences in feed consumption or weight gains of the ewes that could be associated with the various fluorine levels.

These data indicate that there were no differences in the number of lambs born, birth weights of lambs, or average daily gains of lambs, which could be attributed to different levels of fluorine ingestion by the ewes.

The addition of 0.2 percent aluminum sulfate to the ration of sheep on 50 ppm fluorine significantly reduced fluorine storage in the ribs and mandibles. When 0.4 percent aluminum sulfate was added to the ration of sheep receiving 100 ppm fluorine, the fluorine content of the leg, mandible and rib was reduced appreciably.

Since the lambs were kept with the ewes, they consumed some of the feed; therefore, the fluorine content of their metacarpals increased directly with the amount of fluorine added to the different lots.

Laboratory Animals

RATS

Objectives

Numerous experiments have been conducted with both rats and rabbits in an effort to study more intensively some of the factors that might influence, directly or indirectly, the symptomatic changes associated with fluorine toxicosis. Initially these studies were made with weanling rats to determine:

1. The relative toxicity of various fluorine compounds in the Albino rat.
2. The effects of different levels of dietary fluorine as sodium fluoride and the amount of time required to produce detectable gross symptoms of fluorosis.
3. The effectiveness of substances added to the ration for alleviation of the toxic symptoms of chronic fluorosis and bone storage of fluorine.
4. The availability of fluorine from hays grown near industrial plants known to emit fluorine substances.

Experimental Procedure

Highly inbred Albino rats were used in the preliminary investigations to determine the relative toxicity of fluorine in several chemical combinations. Weanling rats 26–30 days of age were maintained in individual cages on basal ration supplemented with the various fluorine compounds to give the calculated fluorine indicated. The basal ration was composed of:

Ingredients	Grams per 100 grams rations
Corn starch	49.0
Alfalfa meal (dehydrated)	30.0
Fat (vegetable)	15.0
Cod liver oil (5 USP units D/gram of feed)	
O.B.I. Salt, Mixture No. 2	3.0
Iodized salt	1.0
Brewers yeast	2.0

This basal ration contained 18.2 percent protein, 10.2 percent fat, 6.5 percent fiber, 0.58 percent calcium, 0.45 percent phosphorus, and 1.5 ppm fluorine.

Distilled water in pyrex flasks was provided *ad lib*. The various hays from effluent areas studied for fluorine availability were ground and substituted for a proportional part of the alfalfa meal in the basal ration. Alleviators to be evaluated were incorporated into the diet at 2 and 4 percent levels in substitution for the corn starch.

Each animal was weighed and checked twice weekly for tooth structural changes. After 30 days on the experimental ration the animals were sacrificed. The tibia, lower mandible, and incisors were cleaned free of tissue and weighed in preparation for total fluorine determination, according to Willard and Winter (1933).

Results and Discussion

The results of this study are summarized in Table 47. The rats receiving fluorine as calcium fluoride stored less fluorine in the bones, and those receiving potassium and sodium fluosilicate stored more than animals receiving fluorine from other sources. Using the 300 ppm level of dietary fluorine as a basis for comparative storage of fluorine from the various compounds, under the conditions of this experiment, storage level from high to low appeared in the following order: Potassium and sodium fluosilicates, potassium and sodium fluorides, rock phosphate, natural and synthetic cryolite, calcium and magnesium fluosilicates, and calcium fluoride. These storage values did not always parallel severity of tooth damage, however.

TABLE 47.—EFFECTS OF FLUORINE FROM DIFFERENT SOURCES UPON BONE FLUORINE CONTENT OF ALBINO RATS

Fluorine source	No. rats	ppm F added to basal ration [a]			
		150	300	600	None
		ppm	ppm	ppm	ppm
Basal ration	28				150
Calcium fluoride	6	——	2020	2220	
Magnesium fluosilicate	4	2350	3890	8530	
Calcium fluosilicate	6	2240	5330	7090	
Synthetic cryolite	6	——	5550	7040	
Natural cryolite	6	6890	5750	8430	
Rock phosphate	4	3430	6140	7160	
Sodium fluoride	8	4630	7060	——	
Potassium fluoride	8	4160	7350	——	
Sodium fluosilicate	6	6620	8750	11640	
Potassium fluosilicate	6	6140	10000	16100	

[a] Experimental ration fed for 30 days.

When fed at the same dietary levels, potassium fluosilicate produced a slightly earlier and more severe tooth effect than did calcium or magnesium fluosilicate, potassium fluoride or sodium fluoride, with little apparent differences in effect between the latter two compounds. Smith and Leverton (1934), however, noted little difference in effects on teeth from any of these compounds. Synthetic cryolite and rock phosphate showed no difference in effects upon the teeth, but effects of both were more pronounced than were those from natural cryolite, which is in agreement with reports by Evans and Phillips (1939).

For the most part, in these young and growing rats, bone storage of fluorine paralleled the dietary concentration up to 300 ppm but, as Schulz (1938) indicated, storage was not always proportional to intake at higher levels.

In consideration of these preliminary investigations and the voluminous literature as reported by Smith (1951) dealing with results from use of sodium fluoride, this compound was adopted as the reference fluorine source for comparison and for the evaluation of biological sources of this element.

Dietary Fluorine Level and Bone Storage: When sodium fluoride was added to the rations of weanling rats to give 15 to 100 ppm fluorine for 30-day feeding periods, bone storage paralleled dietary fluorine concentration. Table 48 demonstrates the dietary-bone concentration relationships

TABLE 48.—EFFECTS OF FLUORINE FROM SODIUM FLUORIDE AND HAY WITH A HIGH FLUORINE CONTENT ON BONE FLUORINE CONTENT OF ALBINO RATS

Dietary F level in ration ppm	No. of rats	Added source of F	F bone storage ppm
Basal	29	None	160
15	6	NaF	260
25	26	NaF	700
30	19	NaF	1150
50	5	NaF	1900
75	34	NaF	3800
100	6	NaF	3300
30	14	Hay [a]	1350
40	5	Hay [a]	1400
50	15	Hay [a]	1670
80	15	Hay [a]	2000

[a] Hay from effluent area incorporated into diet in substitution for alfalfa meal at levels to contain fluorine indicated.

when fluorine was fed as sodium fluoride and as hay from effluent areas. The visible symptoms of fluorosis, as indicated by striations and bleaching of pigments on the lower incisors, were slightly detectable within 15 days at the 25 ppm level, increasing with increased fluorine feeding.

Bone storage of fluorine from ingested hays grown in effluent areas was comparable to, or lower than, amounts of fluorine as sodium fluoride. However, when effluent hays were fed, the teeth appeared to show slightly more wear.

Alleviating Bone Fluorine Storage. The possibility of alleviating the symptoms of fluorosis by feeding a compound which would counteract or remove the fluorine was investigated first with small animals. These studies were pursued along two lines; first an attempt was made to "tie up" the fluorine in the gastrointestinal tract to prevent its absorption from the tract and, secondly, to see whether the rate of removal from body tissues after absorption could be augmented.

Several compounds were evaluated in an attempt to find a suitable alleviator. Those employed were principally aluminum salts, although several non-aluminum compounds also were used. In addition, iodinated casein was used, but it gave no appreciable alleviation, as measured by bone storage of fluorine. Boric acid also was used but proved toxic to the rats at the levels fed.

In these studies weanling rats were maintained on basal ration number 2 which was shown by analysis to contain 10–20 ppm fluorine, 22.8 percent crude protein, 52.7 percent N.F.E., 3.8 percent fiber, 3.1 percent fat, 5.9 percent ash and 88.3 percent dry matter.

The experimental rations for the individual lots were mixed to contain sufficient quantities of sodium fluoride to give the fluorine content indicated in Table 49. Sufficient quantity of alleviator also was added to give the percentage of alleviator indicated. All animals were sacrificed at the end of the six-weeks feeding period. The tibias and femurs were removed and fluorine analyses were run on the bones from each rat. These individual analyses then were averaged to give the lot mean averages included in Table 49.

Three control groups were used with each group of rats fed alleviating materials. One was the basal stock ration, the second had sodium fluoride added to the stock ration at varying levels to determine the extent of fluorine storage, and the third had the alleviators added at varying levels to determine whether there were any effects other than alleviation due directly to the alleviating materials. The results from all lots on the basal ration were combined as were the results from the lots on the stock ration plus various levels of added fluorine but without alleviators. These were then used as the base point to determine the percentage of alleviation secured by the use of various levels of alleviating materials. A negative alleviation figure indicates increased storage over the controls. Many of the treatments were replicated and, in such cases, the results were combined to give a single mean average for the treatment. This is the reason for the varying numbers of animals per treatment. The data presented in Table 49 indicate little alleviation when aluminum sulfate was used at the lower levels.

TABLE 49.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON THE BONE FLUORINE CONTENT OF ALBINO RATS

Alleviator percent	F added in ration ppm	No. animals	Mean [a] in F ppm	S.E.	Range [a] in F ppm	Av. percent increase
0	0	50	283	7.34	180–380	
0	25	4	870	19.42	810–900	307
0	50	26	1473	56.71	1200–2700 [b]	520
0	100	49	2332	74.05	1400–3200	824
0	200	12	3775	126.96	3200–4900	1334
0	400	3	8133	233.61	7700–8500	2874
$Al_2(SO_4)_3 \cdot 18H_2O$						alleviation percent
0.01	0	4	285	6.46	270–300	−0.7
0.01	25	4	858	17.97	830–910	1.4
0.01	50	4	1250	28.86	1200–1300	15.1
0.01	100	4	2225	192.03	2000–2500	4.6
0.01	200	4	4225	165.20	3800–4600	−11.9
0.01	400	4	8625	188.74	8100–9000	−6.4
0.05	0	4	288	17.50	250–330	−1.8
0.05	25	4	638	11.67	620–670	26.7
0.05	50	4	1250	50.00	1100–1300	15.1
0.05	100	4	2125	75.00	2000–2300	8.9
0.05	300	4	3775	149.30	3400–4100	
0.05	400	4	8150	155.45	7700–8400	
0.10	0	4	275	23.62	210–300	2.8
0.10	25	4	690	44.54	610–790	20.7
0.10	50	4	962	14.36	940–1000	34.7
0.10	100	4	1900	40.82	1800–2000	18.5
0.10	200	4	3625	192.03	3400–3900	4.0
0.10	400	4	7225	128.10	7100–7600	11.2
0.50	0	11	175	16.63	120–320	38.2
0.50	50	3	833	13.35	820–860	43.4
0.50	100	11	1164	38.74	1000–1400	50.1
0.50	200	8	1900	65.43	1600–2200	49.7
1.00	0	11	140	7.50	110–170	50.5
1.00	50	3	677	12.04	660–700	54.0
1.00	100	11	865	34.09	730–1100	62.9
1.00	200	8	1500	18.89	1400–1600	60.3
2.00	0	8	91	6.93	70–110	67.8
2.00	100	8	621	24.29	540–740	73.4
2.00	200	8	1250	56.66	1100–1600	66.9
$AlCl_3$ (Sublimed)						
0.50	0	8	176	12.52	140–220	37.8
0.50	50	7	576	40.57	490–800	60.9
0.50	100	8	771	20.38	670–860	66.9
1.00	0	8	165	15.11	120–220	41.7
1.00	50	8	345	19.07	250 [c]–420	76.6
1.00	100	8	499	16.08	400 [d]–540	78.6

[a] Fluorine reported as ppm fluorine in bone ash.
[b] Next highest value 1700 ppm.
[c] Ion exchange resin—courtesy of Rohm and Haas.

TABLE 49.—EFFECTS OF FLUORINE AND FLUORINE PLUS ALLEVIATORS ON THE BONE FLUORINE CONTENT OF ALBINO RATS (*Continued*)

Alleviator percent	F added in ration ppm	No. animals	Mean [a] in F ppm	S.E.	Range [a] in F ppm	Alleviation percent
2.00	0	8	175	21.20	140–310 [e]	38.2
2.00	50	8	242	27.24	180–300	83.6
2.00	100	8	400	21.94	340–530	82.8
$Al_2O_3 \cdot XH_2O$						
0.50	0	5	186	23.54	160–280	34.3
0.50	50	2	1700	0.00	1700–1700	−15.4
0.50	100	3	2933	120.32	2700–3100	−25.8
1.00	0		290	20.84	250–320	−2.5
1.00	50		1667	33.37	1600–1700	−8.1
1.00	100		2700	0.00	2700–2700	−15.8
Amberlite XE—67 [c]						
2.00	0	3	347	17.66	320–380	−22.6
2.00	50	3	1567	33.37	1500–1600	−6.4
2.00	100	2	2650	49.80	2600–2700	−13.6
5.00	0	3	317	6.69	310–330	−12.0
5.00	50	3	1467	33.37	1400–1500	0.4
5.00	100	3	2567	66.75	2500–2700	−10.1
XH 1010 [d]						
0.50	0	8	146	5.65	120–170	48.4
0.50	50	4	730	18.70	690–780	50.4
0.50	100	8	1775	29.12	1500–2200	23.9
1.00	0	8	112	13.01	70–160	61.1
1.00	50	8	650	19.72	610–780	55.9
1.00	100	8	1170	76.77	960–1600	49.8
2.00	0	8	120	17.92	70–220 [e]	57.6
2.00	50	8	500	28.20	410–650	66.1
2.00	100	8	859	71.36	700–1200	63.2
$Al(OH)\ (C_2H_3O_2)_2$						
0.30	0	4	172	22.86	140–240	39.2
0.30	50	4	728	27.50	700–810	50.6
0.30	100	4	1400	40.82	1300–1500	40.0
0.60	0	4	138	16.00	110–170	51.2
0.60	100	4	1055	30.69	970–1100	54.8
0.60						
1.20	0	4	130	18.26	90–170	54.1
1.20	50	4	498	17.02	470–550	66.2
1.20	100	4	858	20.16	800–890	63.2
$MgCl_2 \cdot 6H_2O$						
0.84	0	4	318	21.74	260–360	−12.4
0.84	50	4	1375	85.39	1200–1600	6.7
0.84	100	4	2150	95.74	200–2400	7.8

[d] Aluminum compound containing 72.5% Al_2O_3, courtesy of Dr. Frary, Aluminum Company of America.

[e] Next highest value 150.

The 0.10 percent level shows some alleviation particularly with 25–100 ppm of fluorine added. At the level of 0.5 percent aluminum sulfate the alleviation was approximately 40 to 50 percent; at the level of 1 percent the alleviation was approximately 50–60 percent; and at the level of 2 percent the alleviation was approximately 65–70 percent.

The addition of sublimed $AlCl_3$ resulted in good alleviation. When NaF was incorporated in the ration at levels of 50 and 100 ppm, the aluminum chloride in all cases gave better alleviation than did the aluminum sulfate. On the average, the basic aluminum acetate did not give quite the alleviation of the aluminum sulfate. Both the aluminum sulfate and aluminum chloride are relatively soluble while the aluminum acetate is not soluble in cold water but is soluble in acid.

When the compounds $Al_2O_3 \cdot XH_2O$ and Amberlite were added to the ration little alleviation was obtained. Both of these compounds are relatively insoluble. The compound known as XH 1010 showed some alleviation effect, but generally somewhat less than the aluminum sulfate. The magnesium chloride was added to the ration to see whether magnesium would tie up the fluorine under the acid conditions of the intestine. Work by Werkman and Wilson (1951) indicated that NaF could be used successfully in bacterial studies as a block to the glycolysis cycle, with the formation of a complex magnesium fluoro compound. The alleviation due to the use of magnesium cloride was very slight.

The fluorine stored in the bone increases as the dietary fluorosis increases. This agrees with the report by Jackson *et al.* (1950) which showed a similar relationship when rats were slaughtered at the end of an eight-week period. It will be further noted that the alleviation effect is also, in most cases, a relatively straight line relationship at these levels of fluorine feeding.

The above results agree with the report of Venkataramanan and Krishnasuramy (1948), published while this study was in progress, that certain aluminum salts will decrease the amount of fluoride absorbed from the gastrointestinal tract as reflected by the amount of fluoride stored in the bone.

The alleviation effect produced with the aluminum salts suggested the possibility of removing fluorine already absorbed and stored in the bone. It was recognized that such a possibility was somewhat remote, but an experiment was conducted to determine whether fluoride could be removed from the bone tissue more rapidly than would be expected in normal bone metabolism. Seventy-two rats were fed the basal ration number 2 plus 400 ppm of fluorine *ad lib*. for six weeks. At the end of this feeding period the rats were sub-lotted into eight groups. Group 1 was sacrificed immediately, and the other seven groups were fed the various alleviators at the levels indicated in Table 50.

TABLE 50.—EFFECTS OF ALLEVIATORS AND/OR TIME IN REMOVING FLUORINE FROM RAT BONES HIGH IN FLUORINE

Lot no.	Alleviator percent	No. sacrificed per period	Initial ppm	Weeks from beginning of experiment: 1 ppm [a]	2 ppm [a]	3 ppm [a]	5 ppm [a]	6 ppm [a]	17 ppm [a]	Av. percent decreased initial to 17 weeks
I	Pre-experimental	7	7957							
II	Control	1		6800	5700	7200	5100	5100	3725 [b]	53
III	0.5% $Al_2(SO_4)_3$	1		7400	6400	6000	5700	4500	3825 [b]	52
IV	1.0% $Al_2(SO_4)_3$	1		7700	6800	6000	5700	4700	3550 [b]	55
V	2.0% $Al_2(SO_4)_3$	1		7400	6300	5200	6600	4700	3567 [c]	55
VI	5.0% $Al_2(SO_4)_3$	1		8400	6600	5200	6000	4500	3725 [b]	53
VII	1.0% $AlCl_3$	1		8100	7200	6100	4600	4600	3900 [c]	51
VIII	3.0% Def.	1		7500	5800	6100	4600	4300	4075 [b]	49

[a] Composite of metacarpal and metatarsal bones.
[b] Four animals.
[c] Three animals.

A base line of 7,957 ppm of fluorine in the tibias and femurs at the end of the six-week fluorine feeding period was established. One animal each from Lots 2 through 8 was sacrificed at the end of the first, second, third, fifth, and sixth weeks. The remaining four animals of each lot were slaughtered at the end of the 17th week. Fluorine analyses of the leg bones, made at each of these intervals, is presented in Table 50.

Reason for the noted reduction of fluorine in the bone tissue at the various periods may be an exchange with excretion and a dilution effect due to growth. The animals at the end of the six-weeks fluorine feeding period had an average weight of 152 grams, compared to an average weight of 259 grams at the end of the 17-week depletion period.

The fairly uniform pattern as indicated in Tables 49 and 50 demonstrated little difference in the removal of fluorine from bone tissue by the use of possible alleviators after the cessation of fluorine feeding. The loss of fluorine as indicated in Table 50 is in line with the normal rate of turnover of calcium and phosphate as indicated by Hansard (1954).

Summary

The amount of fluorine stored in the bone of the Albino rat was proportional to the level of fluorine fed, up to 400 ppm of fluorine added, for a 4- and 6-week feeding period.

This work also indicated that four compounds, aluminum sulphate, aluminum chloride, alumina XH-1010, and aluminum acetate will tie up the fluorine and thereby reduce absorption and consequently bone storage when added at levels of 0.5 percent, 1 percent, and 2 percent of the dry ration. The amount of alleviation was apparently related to the amount of soluble aluminum present in the ration.

Once fluorine is deposited in bone tissue none of the alleviating materials used, aluminum sulfate, aluminum chloride, or defluorophos increased removal of fluorine from the bone. The rate of fluorine turnover appears to correspond closely with the rate of calcium and phosphorus turnover.

RABBITS

Objectives

The rabbit is adaptable to pilot fluorine studies of biologically available fluorine because of its low tolerance for fluorine and its ability to consume quantities of roughage. Briggs and Phillips (1952) reviewed the literature on experimental chronic fluorine poisoning in the rabbit and reported growth retardation, joint stiffness, and pronounced tooth and bone structural changes in animals after four months on a natural ration when levels of fluorine above .021 percent (210 ppm) were included in the ration.

The objectives of this phase were:

1. To study the availability to rabbits of fluorine contained in hays from effluent areas.

2. To study symptomatic structural changes in teeth and bones with those evidenced from feeding sodium fluoride.

3. To evaluate the effects of certain alleviating compounds on bone storage of fluorine in the rabbit.

Experimental Procedure

Black Dutch rabbits eight weeks of age, weighing 850 grams, were allotted as indicated in Table 51. They were maintained in wire-bottomed

TABLE 51.—EFFECTS OF FLUORINE AS SODIUM FLUORIDE COMPARED TO HAY GROWN IN EFFLUENT AREAS ON RABBITS

F added in ration ppm	No. animals	Source of F	F consumed Mg. F/kg. body wt.	Av. daily gain gms.	Mean femur bone F ppm [a]	Thyroid wt. gms.
3	6	Basal ration	.02	18	600	1.4
10	6	NaF	.37	16	1300	
20	5	NaF	.71	16	1900	1.5
40	6	NaF	1.48	16	3000	1.6
70	6	NaF	2.22	16	5000	1.7
100	6	NaF	3.60	16	6800	1.9
120	3	NaF	4.28	16	8400	
14	12	Hay_1 [b]	0.48	18	1000	1.7
24	6	Hay_1 [b]	0.83	17	2000	
10	15	Hay_2 [c]	0.34	18	1300	1.9
25	4	Hay_2 [c]	0.81	20	1800	

[a] Percent fluorine was calculated on the dry weight basis.
[b] Represents hay from areas exposed to fumes from aluminum smelting plants.
[c] Represents hay from areas exposed to fumes from phosphate reduction plants.

cages on experimental rations containing known fluorine levels of sodium fluoride and of fluorine from contaminated hays grown near industrial plants known to emit fluorine. Feed consumption and weight records were kept, and distilled water was provided *ad lib.* After they had been on these rations for three months the animals were sacrificed and fluorine analyses were made on the tibia and femur, lower mandible, and incisors. Blood samples were taken for cell pack volume, hemoglobin, and plasma ascorbic acid determinations according to Roe and Kuether (1943). Thyroids were removed and weighed individually.

The basal ration consisted of a concentrate composed of 7 percent yellow corn meal, 38 percent soybean oil meal, and 33 percent skim milk and powder. This was fed as 50 percent of the ration with chopped legume hay twice daily.

Results and Discussion

Table 51 shows that growth was not materially influenced by the levels of fluorine fed. Briggs and Phillips (1952) noted retardation only when dietary fluorine concentration reached .041 percent (410 ppm). At these graded levels of intake the bone concentration, tooth structural changes, and certain physiological variations were used as criteria for measuring response to fluorine ingestion.

The correlation between intake and bone concentration, reviewed by Schulz (1938) for growing animals, is evident in these studies. Bone concentration of fluorine at these levels closely parallels the dietary intake. Bone storage of fluorine from hays from areas near industrial plants emitting fluorine paralleled closely that of sodium fluoride when incorporated into the diet at similar levels. Striations and bleaching of the enamel of the incisors were evident at 20 ppm fluorine level after two weeks. This condition and the time required for its appearance was augmented at the higher levels of dietary intake of fluorine.

Hemoglobin, cell pack volume, and blood plasma ascorbic acid levels were not significantly affected by the added fluorine. However, there was a tendency toward an increase in thyroid weights and a decrease in blood plasma ascorbic acid at the higher levels of fluorine ingestion.

Summary

The symptomatology of fluorine in the rabbit follows a similar pattern to that observed in cattle. The first evidence included structural changes in the incisors; striations, bleaching to chalky white, and increased wear. Bone storage paralleled closely the fluorine intake regardless of source, when time on experiment was standardized.

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